EVALUATING THE DISINFECTION EFFICACY OF LOW-PRESSURE ULTRAVIOLET IRRADIATION ON RIVER WATER

By

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DECLARATION

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ABSTRACT

Irrigation water has been identified as one way by which fresh produce can become contaminated with pathogens. This is a concern in South Africa, where some rivers used for the irrigation of fresh produce often carry pathogens. In this regard, treating river water prior to irrigation is important to reduce the possible risk of foodborne disease outbreaks associated with the consumption of contaminated produce. Ultraviolet (UV) irradiation can be used to decontaminate water and has been shown to be effective against waterborne pathogens. Knowledge gaps, however, still exist regarding the minimum effective UV dose required to effectively inactivate different waterborne pathogens. The aim of this study was, therefore, to evaluate the disinfection efficacy of low-pressure (LP) UV on river water.

Escherichia coli (*E. coli*) strains including three Shiga-toxin producing *Escherichia coli* (STEC) strains were exposed to five different UV doses (20, 30, 40, 50 and 60 mJ.cm^{-2}) in sterile Ringer's solution. Variation in UV sensitivity among strains was observed at all doses. Log reductions ranged between 3.6 - 4.4 log for the lowest dose (20 mJ.cm $^{-2}$). Environmental strains were more UV resistant than the ATCC strain. Based on these results, the influence of water quality on UV irradiation efficacy was investigated by inoculating a resistant environmental STEC strain (STEC 210) into both autoclaved river water and Ringer's solution. Results showed that water quality parameters did not negatively influence UV disinfection efficacy provided the same dose was applied.

The disinfection efficacy of UV (40 and 60 mJ.cm^{-2}) irradiation was then investigated against river water (Eerste, Krom and Plankenburg rivers). The Eerste River showed *E. coli* levels falling below the recommended irrigation guideline limit. The Krom River also showed low *E. coli* levels, but a once-off STEC detection, as well as the consistent occurrence of ESBL producing *Enterobacteriaceae* was observed. The Plankenburg River showed the highest *E. coli* levels and consistent detection of STEC and ESBL producing *Enterobacteriaceae*. UV irradiation of the Eerste and Krom rivers resulted in undetectable levels of *Enterobacteriaceae*, total coliforms and *E. coli.* A targeted three log reduction was achieved following disinfection of the Plankenburg River water at both UV doses. This resulted in water within acceptable irrigation standards. A dose of 40 mJ.cm^{-2} was, however, ineffective against STEC. Repair of all indicator populations was observed and was less than 1% at both UV doses (40

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and 60 mJ.cm^{-2}) but, was less at the higher dose (60 mJ.cm^{-2}). Lastly, the effects of combining pine biochar filtration with UV irradiation was investigated on river water. The combination treatment resulted in improved UVT% and better UV irradiation efficacy of microorganisms.

Overall, UV irradiation showed potential in producing water of acceptable standard for fresh produce irrigation in terms of the *E. coli* load. However, UV efficacy against other important waterborne pathogens such as *Salmonella* was not investigated. It is recommended that, the disinfection efficacy of UV against these pathogens, be investigated in future. Acceptable levels of these pathogens in irrigation water should also be explored, to make guideline recommendations.

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UITTREKSEL

Besproeiingswater is geïdentifiseer as een manier waarop vars landbouprodukte met patogene besmet kan word. Dit is van belang in Suid-Afrika waar sommige riviere wat vir besproeiing van vars produkte aangewend word dikwels patogene bevat. In hierdie verband is dit belangrik om rivierwater voor besproeiing te behandel om sodoende die moontlike risiko van voedseloordraagbare siektes wat met die inname van besmette produkte geassosieer word, te verminder. Ultraviolet (UV) bestraling kan gebruik word om water te ontsmet en daar is gevind dat dit effektief is teen watergedraagde patogene. Kennisgapings bestaan egter steeds ten opsigte van die minimum effektiewe UV dosis wat benodig word om verskillende watergedraagde patogene effektief te inaktiveer. Die doel van hierdie studie was dus om die ontsmettingseffektiwiteit van lae druk (LP) UV bestraling op rivierwater te evalueer.

Escherichia coli (*E. coli*) isolate, insluitend drie Shiga-toksien produserende *Escherichia coli* (STEC) isolate, is blootgestel aan vyf verskillende UV dosisse (20, 30, 40, 50 en 60 mJ.cm^{-2}) in steriele Ringer's oplossing. Variasie in UV sensitiwiteit is tussen isolate waargeneem teen alle dosisse. Log reduksies het gewissel tussen 3.6 - 4.4 log vir die laagste dosis (20 mJ.cm $^{-2}$). Omgewingsisolate was meer UV bestand as die ATCC isolaat. Gebaseer op hierdie resultate is die invloed van waterkwaliteit op UV bestralingseffektiwiteit ondersoek deur 'n UV weerstandbiedende omgewing-STEC isolaat (STEC 210) in beide geoutoklafeerde rivierwater en Ringer's oplossing te inokuleer. Die resultate het gewys dat waterkwaliteit parameters nie UV ontsmettingseffektiwiteit negatief beïnvloed nie mits die regte dosis toegepas is.

Die ontsmettingseffektiwiteit van UV (40 en 60 mJ.cm^{-2}) bestraling is daarna ondersoek in rivierwater (Eerste, Krom en Plankenburg riviere). Die Eerste rivier het *E. coli* vlakke getoon wat voldoen aan die aanbevole besproeiingsriglyn limiete . Die Krom rivier het ook lae *E. coli* vlakke getoon, maar 'n eenmalige STEC teenwoordigheid, sowel as die konsekwente teenwoordigheid van ESBL produserende *Enterobacteriaceae* is waargeneem. Die Plankenburg rivier het die hoogste *E. coli* vlakke getoon asookkonsekwente teenwoordigheid van STEC en ESBL produserende *Enterobacteriaceae*. UV bestraling van die Eerste en Krom Riviere het gelei tot onopspoorbare lae vlakke van *Enterobacteriaceae*, totale kolivorme en *E. coli.* 'n Geteikende drie log reduksie is behaal na behandeling van die Plankenburg Rivier water met beide UV dosisse. Dit het daartoe gelei dat die water

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binne aanvaarbare besproeiing standaarde val. ‘n Dosis van 40 mJ.cm^{-2} was egter oneffektief teen STEC. Die herstel van alle indikatorpopulasies is waargeneem en was minder as 1% by beide UV dosisse (40 en 60 mJ.cm^{-2}), maar was minder by die hoër dosis (60 mJ.cm^{-2}). Laastens is die effek van ‘n kombinasie van denneboom “biochar” filtrasie met UV bestraling op rivierwater ondersoek. Die kombinasie-behandeling het gelei tot ‘n verbeterde UVT% en beter UV bestralingseffektiwiteit teen mikroörganismes.

In geheel toon UV bestraling die potensiaal om water van ‘n aanvaarbare standaard vir vars produk besproeiing, in terme van die *E. coli* lading, te produseer. UV bestralingseffektiwiteit teenoor ander belangrike watergedraagde voedselpatogene, soos *Salmonella,* is nie ondersoek nie. Daar word aanbeveel dat die ontsmettingseffektiwiteit van UV teenoor hierdie patogene in die toekoms ondersoek word. Aanvaarbare vlakke van hierdie patogene in besproeiingswater moet ook bepaal word om sodoende riglyn-aanbevelings te maak.

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CONTENTS

Language and style used in this thesis are in accordance with the requirements of the International Journal of Food Science and Technology. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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ABBREVIATIONS

AEHE	Enteroaggregative haemorrhagic *E. coli*
ARB	Antimicrobial Resistance Bacteria
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BLA	Beta- Lactamase
BPW	Buffered Peptone Water
CA	Clavulanic Acid
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
DBPs	Disinfection By-Products
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
E. coli	*Escherichia coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
FC	Faecal Coliform
ESBL	Extended Spectrum Beta-Lactamase
L-EMB	Levine's Eosin Methylene-Blue Lactose Sucrose
LP	Low-Pressure
LSD	Least Significant Difference
MALDI-TOF	Matrix- Assisted Laser Desorption/Ionisation - Time of Flight
MDR	Multidrug Resistance
MF	Membrane Filtration
MHA	Muller Hinton Agar
MP	Medium Pressure
PCR	Polymerase Chain Reaction
STEC	Shiga-Toxin Producing *E. coli*
TC	Total Coliforms
TDS	Total Dissolved Solids
TSB	Tryptone Soya Broth
TSS	Total Suspended Solids

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USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVT	Ultraviolet Transmittance
VRBG	Violet Red Bile Green
VSS	Volatile Suspended Solids
VTEC	Vero-Toxin producing *E. coli*
WHO	World Health Organisation

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CHAPTER 1

INTRODUCTION

Water is an important component of the environment, without which no life can be sustained on earth (Raji *et al.*, 2015). It is also a raw material for photosynthesis and is, therefore, important for irrigational purposes (Raji *et al.*, 2015). In many parts of the world, however, the availability of good quality irrigation water for agricultural purposes is decreasing (Akpan-Idiok *et al.*, 2012). This is due to the rapid increase in urbanisation, industrialisation, global warming, and drought (Jongman & Korsten, 2017). In South Africa, the availability of water has been threatened by drought spells and pollution (Paulse *et al.*, 2012). In the Western Cape, the recent drought-induced water crisis has resulted in a reduction of water levels in dams (Welch, 2018).

These water scarcity challenges leave farmers with no choice but to explore all possible irrigation water sources (Jongman & Korsten, 2017). While there are many available water sources, surface sources such as rivers are the major source of irrigation in many countries (DWAF, 1996; Steele & Odumeru, 2004; Pachepsky *et al.*, 2011). These sources are, however, classified as the riskiest in terms of microbiological quality (Truchado *et al.*, 2016), because they are subject to various pollution problems (Wimbaningrum *et al.*, 2015). Industrial effluents and discharges of untreated sewage have emerged as the major sources of surface water contamination (Wimbaningrum *et al.*, 2015). As a result, rivers often carry very high microbial loads (Pachepsky *et al.*, 2011; Luyt *et al.*, 2012; Tanaka *et al.*, 2013). In South Africa, *E. coli* levels of up to 6.50 log CFU.mL^{-1} have been reported for the Plankenburg River in Stellenbosch (Paulse *et al.*, 2009; Olivier, 2015). This river, which is also a source of irrigation water for agriculture has also been shown to carry pathogenic *E. coli* (Enteroaggregative and Enteropathogenic) (Lamprecht *et al.*, 2014; Ndlovu *et al.*, 2015).

Pathogenic *E. coli*, and especially Shiga toxin-producing *E. coli* (STEC), has been a leading cause of bacterial infections in both humans and animals and has been implicated in many fresh produce epidemic outbreaks (Heijnen & Medema, 2006; Ram *et al.*, 2011; Beutin & Martin, 2012; Njage & Buys, 2015). An *E. coli* O157:H7 disease outbreak associated with uncooked radish sprouts, claimed the lives of 12 people in Japan in 1996 after 12 000 cases were reported (Michino *et al.*, 1999). In 2005, a large

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outbreak of 135 cases due to verotoxin-producing *E.coli* (VTEC) was reported in Sweden, where lettuce was implicated (Soderstrom *et al.*, 2008). Seven years ago, a multi-national outbreak of enteroaggregative haemorrhagic *Escherichia coli* (EAHEC) resulted in 3 842 cases of human infections and 47 deaths in Europe. The outbreak was caused by contaminated sprouts (Beutin & Martin, 2012). Pathogenic *E. coli* have been reported in surface waters (Ram *et al.*, 2011; Ennis *et al.*, 2012; Odonkor & Ampofo, 2013). Pathogens can be transferred to fresh produce through irrigation with contaminated water.

Another concerning issue about pathogenic *E.coli* is their ability to acquire resistance to antibiotics (Zarfel *et al.*, 2017). Of particular importance, is the resistance mediated by acquired extended-spectrum beta-lactamase (ESBLs) enzymes that can hydrolyse nearly all beta-lactams (Guyomard-Rabenirina *et al.*, 2017). Antibiotic resistance, in particular to third-generation cephalosporins (3GCs) and carbapenems, threatens healthcare globally (Kittinger *et al.*, 2016) because they limit the treatment options available to particular infections (Lenart-Boroń, 2017). These create the need to monitor the occurrence of these bacteria in the environment. Beta-lactamase (*bla*) genes, including CTX-M, TEM and SHV, have been isolated from surface waters in many countries (Figueira *et al.*, 2011; Blaak *et al.*, 2015; Guyomard-Rabenirina *et al.*, 2017; Vital *et al.*, 2018). However, no reports have been made regarding the persistence of these genes in South African Rivers. Antibiotic-resistant bacteria have, however, been isolated from river water samples in South Africa (Olaniran *et al.*, 2009; Romanis, 2013; Lamprecht *et al.*, 2014).

All these health risks become a grave matter when contaminated river water is used to irrigate fresh produce that is intended to be consumed raw. This is due to the possible transfer of microorganisms to the irrigated fresh produce (Lamprecht *et al.*, 2014). On this account, the availability of good quality irrigation water is, therefore, of utmost importance in preventing disease outbreaks and improving the quality of life (Raji *et al.*, 2015). To ensure the safety and quality of irrigation water, regulatory bodies have set up irrigation water guidelines (DWAF, 1996; Allende & Monaghan, 2015). The use of microbial indicators such as *E. coli* has been proposed as a way of characterising the microbial contamination of water (Truchado *et al.*, 2018). The South African Department of Water Affairs and Forestry (DWAF) currently recommends a limit of 1 000 CFU. 100 mL^{-1} (3 log CFU. 100 mL^{-1}) of *E. coli* in agricultural irrigation water (DWAF, 1996).

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In efforts to conform to the guideline limits, effective disinfection methods have been suggested as a means to reduce the microbial load of irrigation water before use. A target reduction of 3 – 4 log units has been suggested by Britz *et al*. (2013) to accommodate for high *E. coli* loads, as previously reported for the Plankenburg River. At present, chemical disinfection methods such as the use of chlorine have been the most preferred water disinfection methods because of the low cost and the ability to leave a disinfection residual (Teksoy *et al.*, 2011). Concerns, however, have arisen in the last decade regarding the formation of carcinogenic disinfection by-products (DBPs) (Freese & Nozaic, 1999; Yaman *et al.*, 2017). In the search to substitute chemical methods, ultraviolet (UV) irradiation has been considered as an alternative (Teksoy *et al.*, 2011). This is because it is easily operated and it effectively inactivates a variety of waterborne pathogens while forming little or no carcinogenic DBPs (Zimmer & Slawson, 2002).

Generally, UV irradiation is generated by either low-pressure (LP) or medium-pressure (MP) mercury vapour lamps (Gayán *et al.*, 2013a). For disinfection to take place, the DNA molecules absorb UV photons between 200 and 300 nm, with peak absorption at 254 nm almost at the peak of germicidal effectiveness for most microbes (Zimmer & Slawson, 2002). The absorption causes damage to the DNA by changing the nucleotide base pairing, thereby forming mutagenic and cytotoxic DNA lesions, which results in cell death (Zimmer & Slawson, 2002).

Despite the advantages of UV disinfection, several factors may influence its effectiveness. A major drawback of UV is the ability of some bacteria to repair the UV induced damage (Morita *et al.*, 2002). There are two routes of DNA repair mechanisms: one is light-independent (dark repair), and the most extensively studied repair occurs in the presence of visible light and is known as photoreactivation or photo-repair (Guo *et al.*, 2009). However, re-growth of microorganisms due to extra nutrients following UV disinfection has also been observed (Sommer *et al.*, 2000). Dark-repair does not greatly influence UV irradiation. In this regard, photo repair, and re-growth of the microorganisms needs attention as they greatly affect the UV disinfection efficacy (Oguma *et al.*, 2002). Another drawback of UV irradiation is that its sensitivity varies between different microorganisms, and even different strains of the same species (Gayán *et al.*, 2013b; Wengraitis *et al.*, 2013). As a result, some microorganisms may show greater UV resistance compared to others, due to differences in both intrinsic and extrinsic parameters (Gayán *et al.*, 2013a). A study

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done by Sigge *et al.* (2016) and Olivier 2015 on different on-farm treatment options to reduce high microbial loads of irrigation water, concluded that environmental *E. coli* strains were more resistant to UV disinfection than *E. coli* reference strains. This illustrates the ability of bacteria to adapt to environmental stress. From their study, the question of whether water quality affects UV disinfection efficacy was raised. Water quality in terms of chemical oxygen demand (COD), UV transmission percentage (UVT%), turbidity, total suspended solids (TSS), volatile suspended solids (VSS) content and conductivity have been shown to negatively affect the UV disinfection effectiveness by obstructing the UV light from penetrating the microorganism (Jones *et al.*, 2014; Zyara *et al.*, 2016).

One of the limitations of the previous research (Sigge *et al.*, 2016; Olivier 2015) was the inability to investigate the effectiveness of UV disinfection of water from different rivers with different physico-chemical properties. Clearly, water quality parameters will influence the disinfection efficiency of UV irradiation. In addition to investigating the UV disinfection efficacy, it is important to also investigate the effect of combining it with a filtration method. This will improve the water quality thereby enhancing UV disinfection. The overall aim of this research, therefore, was to, investigate the LP UV disinfection efficacy on river water. The study focused on; the effects of different UV doses on selected *E. coli* strains; the LP UV disinfection efficacy during the treatment of different river waters, the impact of river water quality and the recovery potential of microorganisms. The occurrence of ESBL producing *Enterobacteriaceae* was also explored. Finally, the effects of combining pine biochar filtration with LP UV irradiation as a means to improve UV irradiation efficacy, was also investigated.

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CHAPTER 2

LITERATURE REVIEW

2.1. BACKGROUND

Water is essential to human life. It also plays a vital role in agriculture and industrial practices (Blignaut & Van Heerden, 2009). Recently, however, population increases, industrialisation and drought have caused a decrease in available water (Sousa *et al.*, 2007). The indications are that human need for water will continue to grow, and it is predicted that 48% of the global population will live in water-stressed catchment areas by 2025 (Blignaut & Van Heerden, 2009). In South Africa, especially Western Cape, record droughts exacerbated by climate change have caused a dramatic water crisis (Welch, 2018). Water levels in the dams supplying drought-stricken Cape Town have dropped to 21.9 % in March 2018. The decline in water levels suggests that residents may have to queue up for the worlds most precious commodity come November (Welch, 2018). The main sources of this scarce resource are ground and surface waters, rain and human wastewater (Steele & Odumeru, 2004). For irrigation purposes, surface waters are the predominant source (Sousa *et al.*, 2007). The surface water is, however, decreasing in quality as many rivers receive discharges of untreated or ineffectively treated domestic and industrial effluent from poorly functioning wastewater treatment plants, as well as informal settlements, which may contaminate the water (Pachepsky *et al.*, 2011).

As a result, surface water is often heavily contaminated with pathogenic microbes (Guchi, 2015). Contaminated surface water that is used for irrigation of fresh produce constitutes a public health risk particularly for products that are intended to be consumed raw (Ijabadeniyi & Buys, 2012; Uyttendaele *et al.*, 2015). This presents the scenario where consumers unknowingly face a high risk of being infected with harmful organisms when consuming fresh produce (Sigge *et al.*, 2016). Although contaminated surface water is a high-risk source, many growers still use surface water for irrigation purposes because it is the most feasible and economic choice (Singh, 2013). This applies especially to growers in rural communities who rely mainly on surface water sources for their daily water needs (Nevondo & Cloete, 1999). In these areas, no wastewater treatment is provided and raw sewage enters the rivers and streams directly (Singh, 2013). Water abstracted from rivers, therefore, is used for irrigation of

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fresh produce often without treatment (Pachepsky *et al.*, 2011). Regarding this, previous research has indicated that irrigation water is one of the main sources of the pathogenic microorganisms found on fresh produce (Cooley *et al.*, 2013). Researchers have also reported that the consumption of contaminated raw fresh produce is a major factor contributing to human gastrointestinal illness (Pachepsky *et al.*, 2011). Blaak *et al*, (2015), reported that the number of produce-related foodborne disease outbreaks are increasing. Increases in foodborne illnesses due to microbial contamination of irrigation water cries for solutions that should be put in place to address the alarming problem. Prevention of river water pollution would be the ideal solution but, it is not feasible (Vergine *et al.*, 2015). Many researchers concluded that reducing microbial contamination of irrigation water before use, is the most effective strategy for the prevention and control of produce contamination (FAO /WHO, 2008). Treating surface water before irrigation is, therefore, increasing as one of the most recommended mitigation responses and prevention strategies for contaminated irrigation water (Vergine *et al.*, 2015). Treated surface water can reduce the risk of pathogen transmission onto fresh produce through irrigation water by inactivating microbial pathogens in the water (Teksoy *et al.*, 2011).

Water treatment methods are typically divided into chemical, physical/mechanical and photochemical techniques. Chlorine, a chemical disinfectant, which is extremely powerful, practical, and cost-effective, have been the most common treatment method used (Profaizer *et al.*, 1997). However, natural organic matter (NOM) present in water sources is a problematic issue for disinfection, because it produces disinfection by-products (DBPs) during chlorination (Yaman *et al.*, 2017). In pursuit of alternatives to chemical disinfection in water treatment, there has been increasing interest in the use of Ultraviolet light because of its excellent biocidal properties without the formation of toxic disinfection by-products (Zimmer & Slawson, 2002). UV radiation is a photochemical method that transfers electromagnetic energy to the microorganisms' DNA, through mercury vapour lamps (Profaizer *et al.*, 1997).

2.2. WATER SCARCITY

Water is a limited resource. It is a key resource in sustaining domestic, industrial and agricultural activities (Blignaut & Van Heerden, 2009). Large volumes of water are required each year, to satisfy the needs of an ever-growing population (Gupta *et al.*,

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2017). The global use of water is expected to rise by 25% by 2030 due to an increase in population from 6.6 billion to about 8 billion by 2030 (Roco *et al.*, 2016). This increase in population and high life standard has increased the scarcity of the world's most limited resource (Sousa *et al.*, 2007). Approximately one billion of the world population lack access to adequate amounts of safe water and rely on unsafe water sources from lakes, rivers and open wells (Guchi, 2015). In noting this, having water of adequate quality and quantity is one of the key requirements for sustainable development (Haddis *et al.*, 2014). Although water scarcity is considered a growing problem worldwide, developing countries are at a higher risk due to lower socioeconomic status and rapid urbanisation which exceeds the capacity of many towns to provide adequate services for their citizens (Haddis *et al.*, 2014). Singh (2013), has described water in South Africa as being either "too little" (due to drought), or "too dirty" (due to pollution). The country is faced with the challenge to either address freshwater shortages or prevent further pollution of the readily available water resources (Jongman & Korsten, 2017). In the Western Cape, the emphasis has shifted to water being "too little" recently.

It is no secret that water is running out in Cape Town and that day zero is a possibility (Welch, 2018). Cape Town's water supplies remain at high risk because the long-term predictions for rainfall in the south-western Cape remain uncertain. Dam levels continue to fall while people are struggling to achieve the city's target of 450 million litres per day (Winter, 2018). With regards to water being "too dirty", the deterioration of the South African surface water resources is another threat the country is facing. Water resources have been under increasing threat of pollution in recent years due to rapid demographic changes, which have coincided with the establishment of human settlements lacking appropriate sanitary infrastructure (Singh, 2013). Bacteriological contamination and pollution of rivers, originating from poorly maintained sanitation facilities, is widespread in the country (Singh, 2013). In terms of the weather, South Africa is a semi-arid, unevenly distributed, water-stressed country, with an average rainfall of about 450 mm, far lower than the global average of 860 mm per year (Wassung, 2010). Weather is negatively impacted by changes in climate (Blignaut & Van Heerden, 2009). In terms of the average "total actual renewable water resources" (TARWR) per person per year, South Africa is the 29th driest country out of 193 countries, with an estimated 1 110 cubic meters (m^3) of water per person (Blignaut & Van Heerden, 2009). It is therefore very challenging to sustain life in Africa

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because, not only does Africa have the highest rate of population growth in the world but, it is also one of the regions that are most vulnerable to climate change. The Intergovernmental Panel on Climate Change (IPCC) predicted an average decline in available water in northern and southern African countries (Haddis *et al.*, 2014).

2.3. SURFACE WATERS

Surface waters include various freshwater sources such as rivers, streams, lakes, and wetlands that are constantly exposed to the relatively high population densities of cities and towns (Shehani & Lui, 2013). Many rural communities in Africa use surface waters (river, boreholes, and wells) as an alternative to pipe-borne water to sustain their daily needs (Abia *et al.*, 2017). Surface waters are susceptible to contamination with pathogenic microorganisms because there are many routes by which microorganisms can enter the water (Pandey *et al.*, 2014). The quality of surface water tends to vary depending on the source, climate and seasonal changes (Sousa *et al.*, 2007). Very often, the surface waters are contaminated by discharges of inadequately treated wastewater, stormwater runoff, as well as livestock or wildlife faeces (Uyttendaele *et al.*, 2015). Rivers have unpredictable water quality since activities upstream can rapidly change the levels of contaminants entering the flowing water (Sousa *et al.*, 2007). River water is the primary source of water for agricultural, domestic and industrial uses and supplies more than 85% of all the water used in South Africa (Sousa *et al.*, 2007). Irrigated agriculture uses more than 60% of the global water and 90% of this share is used by developing countries (Roco *et al.*, 2016). The use of river water for irrigation of fresh produce has, however, been linked to many foodborne outbreaks (Zimmer & Slawson, 2002).

2.4. SOURCES AND TYPES OF SURFACE WATER CONTAMINATION

Surface water such as rivers is often exposed to physical, chemical, and biological contaminants from their proximal environment (Shehani & Lui, 2013). Contamination comes from domestic, industrial and agricultural sources (Pachepsky *et al.*, 2011). Rivers receive discharges of treated domestic and industrial effluent from towns and cities, while return flows from irrigated agriculture contribute additional loads of agrochemicals (Sousa *et al.*, 2007). Water contamination can be categorised as being point or non-point source. Point source contamination (PS) comes from known

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sources like poorly functioning municipal or industrial sewage systems typically associated with manufacturing processes (Monty, 2005). Surface water contamination due to industrial wastes and sewage has been threatening (Kant & Kant, 2010; Paulse *et al.*, 2012). This is because industries discharge a variety of compounds such as heavy metals, wastewater, sometimes in toxic concentrations (Monty, 2005).

Industrial wastes vary depending on the type of industry and location. Some industries generate waste with a high organic matter, and these wastes find their way into the water through industrial discharges. Such industries include dairy and food-processing plants as well as meat-packing houses (Paulse *et al.*, 2012). Other industries, however, generate wastes that are low in organic matter but high in toxic chemicals such as metals, acids or alkalis. These include chemical plants, mining facilities, and textile mills (Alrumman *et al.*, 2016). Non-point source contamination (NPS) is defined as contamination that comes from many different sources rather than from an identifiable, specific point. Contamination can originate from domestic environments such as yards in neighbourhoods or from agricultural production areas such as crop fields (Monty, 2005). Domestic sources are mainly sewage and laundry wastes generated in houses, apartments, and other dwellings (Johnson, 2015). Chemicals, waste products, and soil that are carried by rain into streams or rivers become a part of NPS. Common examples are fertilisers, herbicides, pesticides, spilt motor oil and wastes from pets, wildlife and livestock (Monty, 2005).

2.5. MICROBIAL CONTAMINATION

Microbial contamination is the most harmful type of contaminant. One of the main origins of microorganisms in surface water in developing countries, is poor sanitation (Singh, 2013). Due to improper sanitation in informal settlements, faecal materials are disposed of directly into the rivers through stormwater drainage (Pandey *et al.*, 2014). In urban areas, faecal coliforms are transported to surface waters through the discharge of domestic and industrial wastewater (Johnson, 2015). Polluted surface waters contain a large variety of pathogenic microorganisms including bacteria, viruses and protozoa. The main origin of these pathogenic microorganisms is the faeces of human and warm-blooded animals; which are brought to the aquatic environments through the release of wastewater effluents, surface runoff and soil leaching (Ouattara *et al.*, 2011). It is well established that the risks associated with the

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use of microbially contaminated water for irrigation purposes are a great concern from a health perspective. Traditionally, faecal contamination correlates well with the possible presence of faecal pathogens such as *Salmonella, E. coli* and *Shigella,* as a result, water testing relies on indicator microorganisms for both faecal contamination and the possible presence of disease-causing organisms (Tallon *et al.*, 2005).

Indicator organisms of water contamination

Indicators organisms are microorganisms that have been selected to measure the potential occurrence of faecal material and any associated faecal pathogens (Pachepsky *et al.*, 2011). These organisms are found in the intestinal tract of all warm-blooded mammals, including humans. They can, for this reason, be excreted in the faeces. Indicator organisms are commonly used to assess the microbiological quality of surface waters (Pandey *et al.*, 2014). Due to the large diversity of pathogens in aquatic environments, it is very difficult to detect and enumerate all pathogenic microorganisms present. The low abundance of each species and the absence of standardised and low-cost detection methods make them difficult to enumerate (Singh, 2013). Consequently, routine monitoring and enumeration of indicator bacteria are usually done to evaluate the level of microbial water contamination (Augustyn *et al.*, 2016). A large number of microorganisms have been proposed and tested as indicators (Pachepsky *et al.*, 2011).

These water quality indicators include Total coliforms (TC), Faecal coliforms (FC), *E. coli*, Faecal Streptococci and Enterococci (Singh, 2013). Total coliforms and faecal coliforms were the main organisms used as bacterial indicators for more than a century (Edberg *et al.*, 2000). Coliform levels indicate the hygienic condition of the water and potential risk of infectious diseases. Faecal coliforms, which includes the *E. coli* group, serves as an indication of the level of sewage or faecal contamination, from warm-blooded animals, in a water source (Edberg *et al.*, 2000). Over the years, *E. coli* has become a good choice and it is now widely accepted and considered as a better indicator of faecal contamination of water (Traore *et al.*, 2016). The effectiveness of organisms as indicators is evaluated primarily upon their survival in natural conditions and the cost and skill required for laboratory testing (Mclarnan, 2017). Despite these, Indicators have been criticised because it is shown that bacterial indicators are often poorly correlated with the presence of other microorganisms, such as protozoa and viruses, which can be found in various water sources (Singh, 2013).

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Nonetheless, indicator microorganisms are being used by various authorities such as World Health Organization (WHO) to impose irrigation water quality guidelines (Kant & Kant, 2010). The WHO and the South African Department of Water Affairs (DWAF) recommend water used for irrigation not to contain more than 1 000 *E*. *coli*. 100 mL^{-1} of water (DWAF, 1996; Steele & Odumeru, 2004).

Studies that were done on rivers in South African, however, showed that these rivers often measure more than 1 000 *E*. *coli*. 100 mL^{-1} of water. Paulse *et al.* (2009) reported faecal coliform counts as high as 3.6 x 10^6 *E. coli*. 100 mL^{-1} for the Plankenburg River in the Western Cape. According to a study that was carried out on the Berg and Plankenburg rivers by Britz *et al.* (2013), these rivers used for irrigation in South Africa, fall below the DWAF microbiological standard allowed for irrigated agriculture. Results correlate with the faecal coliform counts observed in previous studies conducted, where water samples collected from the Berg and Plankenburg River systems were also analysed for the faecal indicator groups (Ndlovu *et al.*, 2015). In the Berg and Plankenburg River systems, all faecal coliform counts obtained exceeded the guidelines of 1 000 *E*. *coli*. 100 mL^{-1} stipulated by DWAF for irrigation water (Ndlovu *et al.*, 2015). Apart from rivers in the Western Cape, some rivers in other provinces also measure more 1 000 *E*. *coli*. 100 mL^{-1} of water. In 2003, the Jukskei River in the Gauteng Province measured 1.1 × 10^6 cfu.100 mL^{-1} of *E. coli*, while the Umungeni River was contaminated with 1.1 × 10^6 cfu.100 mL^{-1} of *E. coli* (Ijabadeniyi & Buys, 2012).

Escherichia coli as an indicator of water contamination

Esherichia. *coli* is a bacterial species that is widely used and accepted as an indicator organism in the testing of water quality all over the world (Ndlovu *et al.*, 2015). *E. coli* is almost exclusively a faecal microorganism and constitutes over 90 % of the coliform flora of the human intestine (Guchi, 2015). Monitoring of *E.coli* levels is adopted in most European regulations as a basic tool to assess the microbiological quality of river water (Vergine *et al.*, 2015). The evolution of *E. coli* as the predominant indicator organism dates back many years. It was first studied and proposed as the primary water indicator by Theobald Smith in the 1890s (Edberg *et al.*, 2000). In 1893, it was introduced as an indicator of faecal contamination of water quality (Tallon *et al.*, 2005). *Esherichia. coli* has since then proven to be a better indicator of faecal contamination of water supplies than total and faecal coliforms (Traore *et al.*, 2016).

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The presence of non pathogenic *E. coli* provides evidence of an increased likelihood of potential contamination of water by ecologically closely related pathogens (Uyttendaele *et al.*, 2015). If not detected, the water is regarded as free from faecal contamination (Teksoy *et al.*, 2011). The key attributes that led to its use as the preferred indicator include, its ability to be present at high concentrations in human faeces and its significant correlation with gastrointestinal disease (Mclarnan, 2017). Many studies have shown that *E. coli* are the only coliform organism that is commonly found in the gastrointestinal tract of humans and warm-blooded animals (Traore *et al.*, 2016). While *Klebsiella, Citrobacter* and *Enterobacter* have been isolated from human faecal samples, their presence was in small numbers (Edberg *et al.*, 2000). Moreover, according to Vergine *et al*. (2016) *E. coli* does not replicate appreciably outside of its natural habitat, therefore in non-enteric environments, its population rapidly tends to decrease after an event of faecal contamination (Vergine *et al.*, 2015). These, coupled with improved detection methods for *E. coli*, have contributed to the selection of *E. coli* as a more reliable indicator of water quality (Odonkor & Ampofo, 2013). Numerous studies have also shown that detection methods available for *E. coli* are more accurate, specific and sensitive than those for thermotolerant coliforms (Tallon *et al.*, 2005). Although *E. coli* have been accepted by many nations as an indicator of faecal contamination, there have also been different opinions.

In the mid-2000s, a body of research began to indicate that *E. coli* might have properties that result in false positives in faecal contamination testing (Mclarnan, 2017). Research conducted by Teagasc (2010), found that *E. coli* can be integrated into the indigenous microbial community in soils and survive for more than nine years, suggesting that, the presence of *E. coli* in surface or groundwaters may not necessarily be indicative of recent faecal contamination. It is also well recognised that *E. coli* might not be a suitable indicator of some specific enteric pathogens and that its concentrations are often poor predictors of the potential for water to cause gastrointestinal illness because it is not correlated with the presence of pathogens such as *STEC* or *Salmonella* spp (Tallon *et al.*, 2005; Pachepsky *et al.*, 2011). The Canadian Federal Provincial Territorial Committee stated that *E. coli* are also not a good indicator for parasites such as *C. parvum*, *G. lamblia*, *Y. enterocolitica* or enteric viruses (Tallon *et al.*, 2005). Therefore, the absence of faecal indicator bacteria does not necessarily imply that pathogens are absent (Edberg *et al.*, 2000). This suggests

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that *E. coli* levels might be insufficient in predicting the risk of exposure to certain pathogens accurately (Pachepsky *et al.*, 2011).

Shiga Toxin-producing Escherichia coli *(STEC)*

Esherichia. coli has become notorious over the years because of some strains that are pathogenic to humans (Traore *et al.*, 2016). These strains have been grouped, based on epidemiological evidence, phenotypic traits, clinical features of the disease and specific virulence factors (Heijnen & Medema, 2006). The six *E. coli* pathotypes that carry virulence genes includes, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC, including O148), Enterohaemorrhagic *E. coli* (EHEC) also known as STEC (including O157), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC, including O124) and diffusely adherent E. coli (DAEC) (Traore *et al.*, 2016). STEC is a distinct class of enteric pathogenic bacteria that is capable of causing a spectrum of human illnesses (Cooley *et al.*, 2013). Illnesses range from moderate diarrhoea to severe bloody diarrhoea termed haemorrhagic colitis (HC) and haemolytic-uraemic syndrome (HUS) (Heijnen & Medema, 2006).

The ability of STEC to cause human diseases is due to the fact that STEC produces one or more Shiga toxin genes (*Stx1, Stx2* or other variants). These genes inhibit protein synthesis of host cells leading to death (Cooley *et al.*, 2013). Human pathogen STEC can also carry the chromosomal gene *eae* which encodes an adhesion called intimin. Additional virulence factors have also been described in the STEC strains (Lascowski *et al.*, 2013). Although many STEC serotypes have been described, O157:H7 is the serotype that is mostly associated with many disease outbreaks in different countries (Lascowski *et al.*, 2013). STEC can survive in soil, pasture and water and transmitted through raw or undercooked meat, unpasteurised milk, contaminated vegetables as well as contaminated water (Heijnen & Medema, 2006; Mclarnan, 2017). Physical contact with places where cattle have previously grazed and recreational water sources can, therefore, result in STEC infection (Thorpe, 2004). An increasing number of STEC outbreaks are associated with the consumption of contaminated fresh produce (Cabral, 2010). Minimal exposure to STEC can result in disease not only because of this mode of STEC transmission but also due to the very low infectious dose (<100 organisms) of some strains (Thorpe, 2004).

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Antimicrobial resistance

Antimicrobials in water come from many different sources such as hospital effluents, informal communities, industries and farms (Kittinger *et al.*, 2016). While it is normal for bacterial species to acquire antimicrobial resistance in a clinical setting, the wide use of antimicrobials in different practices has resulted in the evolution and spread of antimicrobial resistant (AMR) bacteria outside clinical settings (Ye *et al.*, 2017). As a result, the possibility of getting exposed to AMR bacteria outside a healthcare setting has increased (Blaak *et al.*, 2015). Antimicrobial resistant bacteria (ARB), antimicrobial resistance genes (ARG) and diluted antimicrobials have been found in various aquatic environments including rivers and lakes (Miranda *et al.*, 2016). The aquatic environment plays a significant role in the spread of antimicrobial resistance because, surface waters are the main receptacle of industry and hospital discharges (Goñi-Urriza *et al.*, 2000). Surface waters not only act as habitat, and transport systems but also as a "marketplace" for microorganisms, where susceptible strains can acquire new resistance genes (Kittinger *et al.*, 2016). While noting, that rivers are one of the major sources of water for human use, discharges may contribute to the spread of bacterial antimicrobial resistance in the general population and environment (Goñi-Urriza *et al.*, 2000). Exposure to AMR bacteria can be through preparation and consumption of contaminated food products, or contact with contaminated surface water (Blaak *et al.*, 2015; Ye *et al.*, 2017).

There are several mechanisms by which different bacteria develop antimicrobial resistance (Guyomard-Rabenirina *et al.*, 2017). The most common in *Enterobacteriaceae* is the production of beta-lactamase enzymes, which hydrolyse the beta-lactam ring structure of certain antibiotics. The extended spectrum β-lactamase (ESBL) and plasmid-mediated *cephalosporinase* (AmpC) producing isolates constitute a particularly important branch in this lineage (Ye *et al.*, 2017).

ESBL Producing *Enterobacteriaceae*

Extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* are bacteria that produce enzymes capable of hydrolysing the β-lactam ring structure of an antibiotic (penicillin, aztreonam, and first, second, and third-generation cephalosporins), rendering it inactive (Figure 1) (Leavell, 2016; Guyomard-Rabenirina *et al.*, 2017). These enzymes confer resistance to most beta-lactam antibiotics

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including, penicillin, broad-spectrum (first, second, and third-generation) cephalosporins and monobactams, which reduces treatment effectiveness for infections caused by these bacteria (Blaak *et al.*, 2014). These strains are, however, often susceptible to cephamycins and carbapenems (e.g. imipenem, meropenem) (Rupp & Fey, 2003). Most ESBLs enzymes also confer resistance to fourth-generation cephalosporins (such as cefepime or cefpirome) (Ye *et al.*, 2017). Extended-spectrum β-lactamase genes are typically found encoded on bacterial plasmids that can easily be transferred between bacteria from the family *Enterobacteriaceae* (Leavell, 2016). There are different types of β-lactamase enzymes, coded by different types of *bla* (β-lactamase) genes. These enzymes are grouped into families, based on genetically similar characteristics (Shehani & Lui, 2013). Many of them are members of TEM and SHV β-lactamase families, but other groups, such as CTX-M, OXA, and PER, VEB and GES β-lactamases have also been described (Ye *et al.*, 2017; Zarfel *et al.*, 2017). Two decades ago, TEM and SHV were the most prevalent ESBL enzyme families in nosocomial and community settings worldwide (Ye *et al.*, 2017). However, these groups have been replaced by CTX-M since the early 2000s, but they are still frequently detected (Guyomard-Rabenirina *et al.*, 2017; Zarfel *et al.*, 2017).

The first description of ESBL producing Enterobacteriaceae was isolated from hospitalised humans, many nosocomial outbreaks and later, community-associated infections have been reported worldwide (Zurfluh *et al.*, 2013). According to recent studies, *E.coli* has become the most predominant species among ESBL producing *Enterobacteriaceae* and have been reported to be present in wastewater, surface water, sewage, and sediment samples (Blaak *et al.*, 2014). Surface waters are considered to be of special importance as a reservoir of resistance genes since they are recipients of bacteria from different sources (Zurfluh *et al.*, 2013). Shehani and Lui (2013), found a high occurrence of ESBL producing bacteria in local surface waters. In their study, a total of 19 isolates were found, possessing at least one of the *bla* genes tested for. Extended-spectrum β-lactamase producing *Enterobacteriaceae* have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control issues (Rupp & Fey, 2003). Infections associated with ESBLs vary from minor conditions such as urinary tract infections to more severe conditions such as pneumonia and bacteraemia. Human exposure to ESBL producing strains in rivers may, therefore, prove to be hazardous (Shehani & Lui, 2013).

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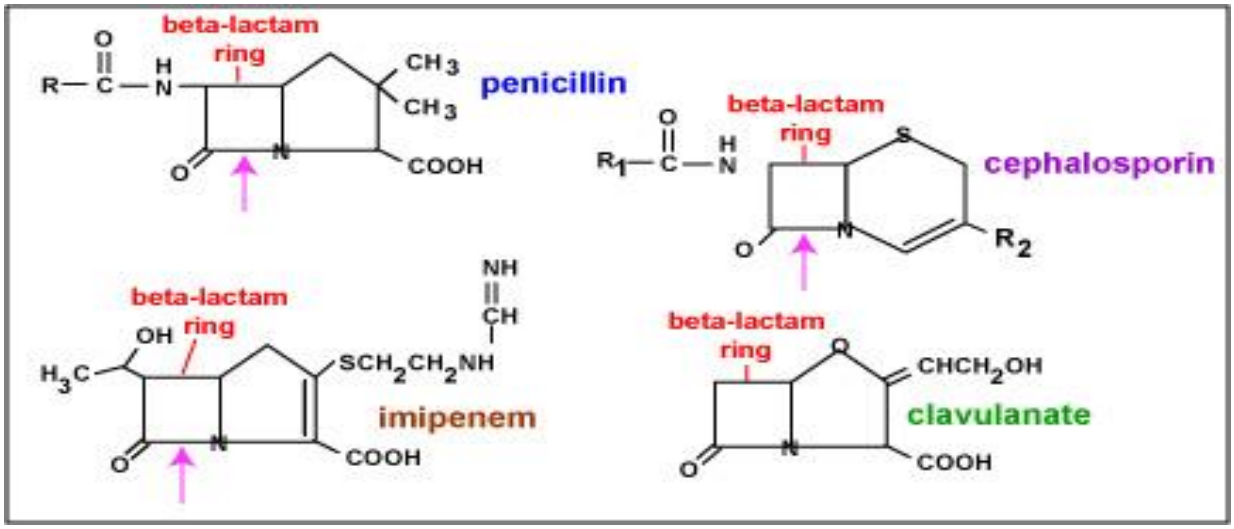

Figure 1 Schematic representation of Beta-Lactam antibiotics (Kim & Cho, 2010).

2.6. FOODBORNE OUTBREAKS DUE TO CONTAMINATED IRRIGATION WATER

Foodborne illnesses are caused by different pathogens. Each pathogen manifests itself in a unique way. For some, illnesses are likely to be mild with no lasting effects. For others, the corresponding illness is characterised by a high hospitalisation and death rate (Scharff, 2010). Four specific human pathogens, *E. coli* O157:H7, *Salmonella*, *Cyclospora*, and *Hepatitis* A virus, have accounted for 96% of the outbreaks and 95% of the illnesses in reported produce-related outbreaks from 1996 to 2007 (Bihn *et al.*, 2013). There has been evidence that indicates that contaminated irrigation water is an important source of foodborne pathogens on fruit and vegetables (Britz *et al.*, 2013). Duffy *et al*. (2005) proved that irrigation water is indeed the leading pre and post-harvest source of contamination of produce when they isolated a total of 22 *Salmonella* strains found in environmental samples (irrigation water, soil, packing shed equipment).

Their results showed that 16 isolates were from irrigation water and six from the packing shed types of equipment. Countries worldwide have reported problems associated with pathogens in fresh produce (FAO/WHO, 2008). Intake of fresh or raw vegetables (especially those used for salads) and fruits have been associated with foodborne diseases (Oloyede & Centre, 2010). Other reported foodborne disease outbreaks associated with fresh produce have been linked to irrigation water (Jongman & Korsten, 2017). Foodborne diseases (i.e., diarrhoea, gastrointestinal

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illness) caused by various bacteria have been the cause of many outbreaks (Pandey *et al.*, 2014). Irrigation water was implicated in the outbreaks of *E. coli* O157:H7 infection from contaminated lettuce (Steele & Odumeru, 2004). The majority of foodborne pathogen outbreaks reported originated from commercial farming systems, indicating that outbreaks from small-scale farming and homestead gardens are underreported (Jongman & Korsten, 2017). Incidences of foodborne pathogens on fresh produce vary by region and can be extremely high in some developing countries (Pachepsky *et al.*, 2011). In the United States, the number of foodborne illness outbreaks linked to fresh produce has increased over the last years (Harris *et al.*, 2003). A total of 350 outbreaks of *E. coli* O157:H7 were reported in the United States; 52% and 9% were caused by foodborne or waterborne sources, respectively, between 1982 and 2002 (Cooley *et al.*, 2013).

In 2005, a lettuce-associated outbreak of *E. coli* O157:H7 infection affected 135 people in Southwestern Sweden. Contaminated irrigation water was the likely source of infection (Edelstein *et al.*, 2014). Irrigation water was reported as a likely source of contamination of fresh green salad that was implicated in another 2013 *E. coli* O157:H7 outbreak in Sweden. This resulted in 19 reported cases (Edelstein *et al.*, 2015). In the Montana, USA, outbreaks of *E. coli* O157:H7 were associated with the consumption of green salad that was contaminated due to unsafe irrigation water (Pachepsky *et al.*, 2011). The third largest and deadliest *E. coli* outbreak reported in history occurred in 2011, where more than 3 800 cases resulted from a single outbreak. This was associated with sprouts and caused the death of at least 54 people in Europe (Jongman & Korsten, 2017). In 2013, an outbreak control team in Sweden investigated the source 19 reported cases of an enterohaemorrhagic *E. coli* outbreak that was associated with contaminated fresh salad. The investigation showed that contaminated irrigation water was the likely source of the outbreak (Jongman & Korsten, 2017). Another *E. coli* O157:H7 outbreak linked to pre-packed spinach that was reported in the United States of America also concluded that irrigation water was linked to pathogens on fresh produce after the outbreak strain was recovered from river water used for irrigation (Jongman & Korsten, 2017). In Japan, about 10 000 people were affected by an *E. coli* O157:H7 outbreak that was linked to consumption of radish sprouts. Again irrigation water was the source of contamination (Steele & Odumeru, 2004). These outbreaks emphasise the detrimental effect that contaminated irrigation water can have on human health.

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2.7. WATER TREATMENT

The increase in foodborne outbreaks due to contaminated irrigation water evidenced by a number of publications demonstrates the need to address the problem as a whole (Pandey *et al.*, 2014). Treatment is usually applied to improve the quality of water by reducing the microbial load of the water (Yaman *et al.*, 2017). There are currently several methods available for the reduction of microorganisms in water (Caslake *et al.*, 2004). These treatment methods can generally be classified as being chemical, physical/mechanical or photochemical in nature (Jacobs *et al.*, 2006). Not all treatment methods are suitable for surface water sources due to the complexity and variability of some technologies. Filtration, chlorination, ozonation and ultraviolet irradiation, can all potentially reduce the levels of microorganisms in irrigation water (Steele & Odumeru, 2004). Chemical methods such as the use of chlorine have been a standard water disinfecting method for many years (EPA, 2013). However, disadvantages such as the formation of disinfection by-products (DBPs) and resistance of certain microorganisms such as *Cryptosporidium* has led to a search for alternative methods (Freese & Nozaic, 1999; Mwabi *et al.*, 2013). In recent years, advanced oxidation processes (AOPs) have received considerable attention for the disinfection of water and wastewater. Amongst them, greener technologies such as UV irradiation have emerged because of several advantages, such as high disinfection efficiency with most viruses, bacteria and protozoa, no unidentified toxic DBPs and safe operation (Guo *et al.*, 2009).

Chemical treatment methods

Chemical treatment involves the use of chemicals to improve the quality of water (Buchanan, 1985). This method relies on the interactions of chemicals with water contaminants (Shah *et al.*, 2015). Chemicals can either be used as stand-alone technologies, or in combination with other treatment methods (Teksoy *et al.*, 2011). Chemical disinfectants such as chlorine, ozone, peracetic acid, and hydrogen peroxide have been commonly used for water disinfection because of their low cost, ease of handling, and their ability to provide disinfectant residual (Teksoy *et al.*, 2011).

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Chlorination

Introduction, mode of action, advantages and disadvantages

Chlorine is a strong oxidising chemical that is used to kill bacteria (Mounaouer & Abdennaceur, 2012). It comes in many different forms including chlorine gas (most common), chlorine dioxide, hypochlorite (bleach) (Freese & Nozaic, 1999). Chlorine's use as a water disinfectant dates back to over a century ago and has been credited with saving a significant number of lives worldwide (Freese & Nozaic, 1999). Globally, it remains the most widely used chemical disinfectant in water treatment for both primary disinfection and for the maintenance of a residual in distribution systems (EPA, 2013). The main reasons for this are the high reliability of the bactericidal effects and the ability to remove iron and manganese from water (Freese & Nozaic, 1999). Chlorine also provides disinfectant residual (Teksoy *et al.*, 2011). It is relatively easy to handle and the capital costs of chlorine installation are low and are simple to dose (Freese & Nozaic, 1999). It has in spite of that, received a lot of negative publicity over the past few decades (Freese & Nozaic, 1999). The discovery in the 1970's that treating water with chlorine could result in the formation of potentially harmful disinfection by-products (DBPs) caused international concern and these resulted in authorities reviewing chlorination practices in order to minimise DBP formation (Steele & Odumeru, 2004).

Many treatment plants that use chlorine as a primary disinfectant fail to achieve desired microbial inactivation levels without the formation of DBPs that often exceed regulatory levels (Chauret *et al.*, 2001). This is because, chlorine reacts with naturally occurring organic matter and other precursors in water to form carcinogenic disinfection DBPs (Teksoy *et al.*, 2011). Exposure to DBPs could contribute to many health problems such as asthma, cancer, fertility problems, heart disease, eczema and birth defects, in addition to the unpleasant smell and taste of chlorinated water (Mounaouer & Abdennaceur, 2012). Another disadvantage of chlorine is that it is ineffective to some epidemic microorganisms (Morita *et al.*, 2002). Even though chlorine is a reliable disinfectant, its reaction with organics on the cell wall is often limited and therefore, not effective in removing certain pathogens of concern such as *Cryptosporidium* and *Giardia* at low doses (Clarke & Bettin, 2006). These parasites are pathogenic to humans and can be transmitted through the water supply. Outbreaks of *Cryptosporidium* infection have been reported in many countries (Oguma *et al.*,

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2001). In addition to the disadvantages, the effectiveness of chlorine depends on the chemistry of the water (Parke & Fisher, 2012). For example, the sanitising activity of chlorine is strongly dependent on pH. The optimal pH range for water treated with hypochlorite is 6.0-7.5 (Parke & Fisher, 2012). Moreover, treatment chemicals are also costly to manage in rural water systems. Due to lack of availability in rural areas, the transportation costs of importing chemicals can be a major concern for small systems. Not only that, but the use of chemicals requires monitoring from skilled personnel, as the chemical dosing-process is highly sensitive to fluctuations in raw water quality such as pH (Guchi, 2015).

Ozonation

Introduction, mode of action, advantages and disadvantages

Ozone is an unstable form of oxygen, which is broken down to oxygen stable molecules and oxygen atoms with a high oxidation potential (Vaju *et al.*, 2008). Ozone has been widely used and recognised especially in Europe, as one of the most effective disinfectants for water, as an alternative to chlorination (Li *et al.*, 2011). It is the only chemical that has the ability to effectively inactivate *Giardia* or *Cryptosporidium* (EPA, 2013). Apart from that, ozone can also be used in water treatment to remove organic micropollutants, taste, odour, and colour (Yaman *et al.*, 2017). In like manner, ozone exerts a disinfection residual, but this dissipates rapidly. It is, therefore, necessary to use another disinfectant with a longer lasting residual to ensure no regrowth occurs in the distribution system (Freese & Nozaic, 1999). Although ozonation systems are good to remove colour, odour, and taste, they have disadvantages such as high operational costs. This is due to the fact that ozone must be generated on site and that it requires highly skilled staff in order to operate and maintain the ozone plant (Freese & Nozaic, 1999).

In addition, an overdose of the ozone gas generated by this form of water treatment can harm not only downstream water distribution systems, but humans as well, and must, on these grounds, be carefully monitored (EPA, 2013). The ozonation process also leaves a residual ozone level that could be harmful and must be removed for operator/user safety (IWG, 2002). Despite the fact that interest in ozone was aroused due to concerns over DBPs formed during chlorination, it has since been discovered that ozone has a similar drawback as that of chlorine (Biryukov *et al.*,

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2005). It also reacts greatly with organic matter in the water resulting in the formation of DBPs, especially non-halogenated DBPs such as aldehydes, ketones and carboxylic acids, many of which have been found to be mutagenic or carcinogenic (Biryukov *et al.*, 2005). Ozone also gives rise to the formation of bromates when bromide is present in the water (Freese & Nozaic, 1999). Furthermore, it is difficult to control the ozone dosage levels making this method an unpredictable disinfectant (IWG, 2002). For this reason, ozone treatment is usually backed up by chlorination, with the accompanying drawbacks of chemical addition and removal (Freese & Nozaic, 1999).

Peracetic Acid (PAA)

Introduction, mode of action, advantages and disadvantages

Peracetic acid (PAA) is a strong oxidising agent commonly used as a disinfectant in the food, beverage and paper industries (De Velásquez *et al.*, 2008). It has been considered and has gained interest in water treatment because of its strong bactericidal properties (Profaizer *et al.*, 1997). Peracetic acid was found to be an effective disinfectant, generally providing disinfection comparable to that obtained when using chlorine at equivalent mass concentrations (Freese & Nozaic, 1999). It oxidises the outer cell membrane of bacterial cells by disrupting the function of the lipoprotein cytoplasmic membrane (De Souza *et al.*, 2015). A notable advantage of PAA is its high stability. It has been reported in many studies to be biodegradable and very soluble (De Velásquez *et al.*, 2008). Also, unlike other chemical disinfectants, PAA produces insignificant amounts of mutagenic DBPs (De Velásquez *et al.*, 2008). Up to date, there are no reports pointing to PAA as being carcinogenic or that it presents toxicity in the reproduction and human development (De Souza *et al.*, 2015). Other advantages include: being easy to implement (without the need of high investment), the large spectrum of microbial activity even in the presence of heterogeneous organic matter (Profaizer *et al.*, 1997). Peracitic acid also provides disinfectant residual (Freese & Nozaic, 1999). Despite the many advantages, PAA is not readily available in Southern Africa at present, and therefore not an economically feasible option at this stage (De Souza *et al.*, 2015). There are also risks associated with the handling of the chemical (Freese & Nozaic, 1999).

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Hydrogen peroxide (H_2O_2)

Introduction, mode of action, advantages and disadvantages

Hydrogen peroxide (H_2O_2) is a long-lived and efficient oxidant. It has over the past two decades, attracted attention as a water disinfection method due to evident benefits in the disinfection results (De Velásquez *et al.*, 2008). It is widely known for its sterilant and antiseptic properties (Pettit, 2014). Hydrogen peroxide, on its own, is a mild disinfectant agent, achieving little or questionable inactivation of bacteria and viruses (EPA, 2013). On account of these, it has not been widely used as a sole disinfectant for water treatment (Teksoy *et al.*, 2011). Its disinfection capacity has, however, been improved when used in conjunction with other methods (Teksoy *et al.*, 2011). Its use in combination with ozone and ultraviolet light produces increased results (EPA, 2013). Just like any other chemical disinfectant, H_2O_2 also, reacts with organic matter in the water, yielding very harmful DBPs (Freese & Nozaic, 1999). It is unstable when used in water treatment. Its use in water treatment has therefore been very limited due to the instability in storage and the difficulty in preparing concentrated solutions.

Physical treatment methods

Physical treatment includes physical activities that do not allow gross chemical or biological changes to take place (Turtoi, 2013). This process involves the separation of suspended and colloidal particles from water (Huisman & Wood, 1974). Physical treatment methods were one of the earliest methods used to remove solids from water (Lozano *et al.*, 2004). Water passes through screens to remove debris and solids. Heavy solids settle out from water by gravity while, particles with entrapped air float to the top of the water (Lozano *et al.*, 2004). These physical processes are employed in many modern water treatment facilities today (Lozano *et al.*, 2004). The most common physical treatment methods include the use of slow sand filtration and filtration membranes (Huisman & Wood, 1974).

Slow sand filtration

Introduction, mode of action, advantages and disadvantages

The slow sand filtration process provides treatment through physical filtration of particles and removal of pathogens and organics from water (Guchi, 2015). It has for

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more than a century been used for preventing the spread of gastrointestinal diseases (Hendricks, 2006). The first slow sand filter was installed by James Simpson in 1829 (Huisman & Wood, 1974). At the time, the main purpose was to reduce suspended solids and turbidity in water. Its disinfection capabilities were then realised later (Huisman & Wood, 1974). The process basically integrates physical and biological process together to remove contaminants from water (Verma *et al.*, 2017). The slow sand filtration system is generally made up of the supernatant water layer, sand bed (fine and coarse sand), gravel and outlet hose (Figure 2) (Guchi, 2015). The upper biologically active layer of the sand bed is known as a biofilm (Schmutzdecke) (Huisman & Wood, 1974). During disinfection, the water slowly passes through a bed of porous material (Guchi, 2015). The Schmutzdecke removes natural organic matter, transforms synthetic organic compounds, retains pathogens and produce microbiologically safe water (Allende & Monaghan, 2015). Biological removal of nitrate or other organic contaminants is also done by the Schmutzdecke (Verma *et al.*, 2017).

Water treatment efficiencies of slow sand filtration have been about 99% effective for removal of turbidity, suspended solids, and waterborne pathogens (Huisman & Wood, 1974). It also has the potential of removing cysts of *Giardia* and *Cryptosporidium* enteroparasites (Verma *et al.*, 2017). Aside from disinfection efficiency, slow sand filtration is a less energy-intensive technology, with lower dependency on chemicals and skilled labour (Verma *et al.*, 2017). Thus, eliminating the risks associated with the use of chemicals (Guchi, 2015). Despite the fact that slow sand filtration has proven to effectively remove pathogenic bacteria, protozoa, viruses, suspended solids and even reduce turbidity levels, its effectiveness depends on physical and operational characteristics of the filter such as size, bed depth, filtration rate, biological maturity of the filter, and cleaning practices (Guchi, 2015). Also, slow sand filtration requires good maintenance as the thickening Schmutzdecke needs to be changed from time-to-time to maintain sufficient flow of water in the system. Adding to this, the filtration process is ineffective in removing nematodes as a result of the large pore size of the sand bed (Allende & Monaghan, 2015). Slow sand filters occupy large surface areas and are associated with high installation expenses (Huisman & Wood, 1974).

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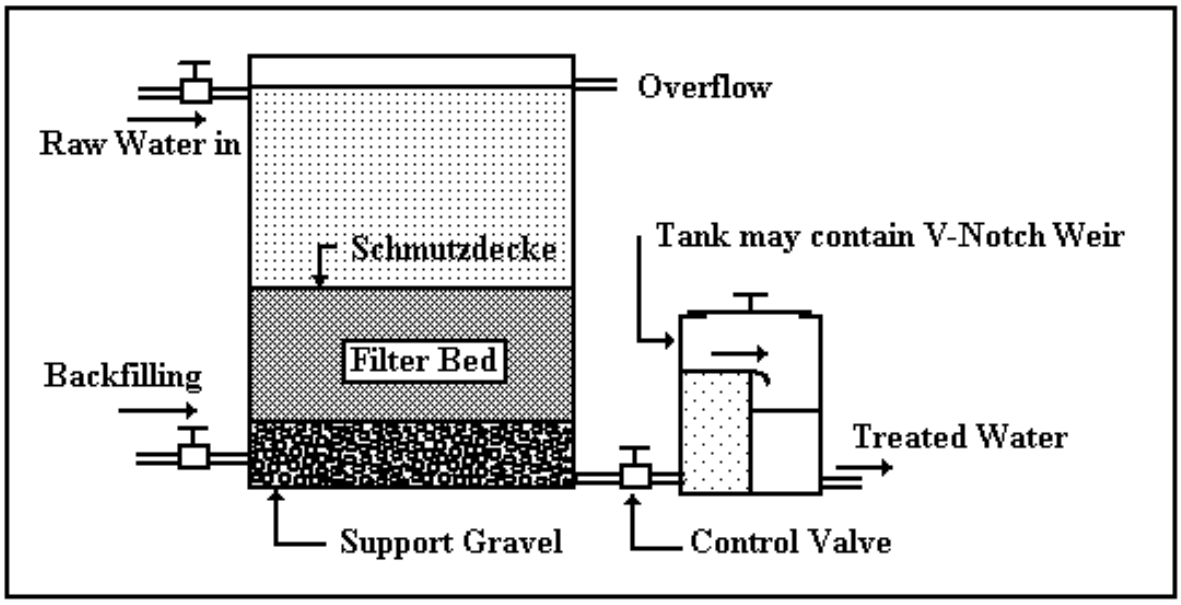

Figure 2 Schematic representation of a slow bed sand filter adapted from (Huisman & Wood, 1974).

Membrane filtration

Introduction, mode of action, advantages and disadvantages

Membrane technology is increasingly becoming popular for the treatment of water (Sadr & Saroj, 2015). For the past decade, membrane filtration systems have been used globally to remove microorganisms, particulates, and natural organic material from water (Sadr & Saroj, 2015). They consist of a wide range of processes depending on the desired quality of the effluent. They are classified based on effective size range. Most common membrane processes used in water treatment include membrane microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) (Minnesota Rural Water Association, 2001). Microfiltration is a separation process that removes larger particles and colloidal substances from water (Sadr & Saroj, 2015). Because of the big pore size (Table 1), microfiltration does not provide a barrier against smaller particles such as bacterial species (Minnesota Rural Water Association, 2001). It is, therefore, combined in a series of multi-stage filters to achieve more highly treated effluent quality (Gurol, 2011). Ultrafiltration is a low pressure driven, separation process, that allows water and substances with low molecular weight to move through a porous membrane (Sadr & Saroj, 2015). This system

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provides an effective barrier for bacteria, viruses, suspended particles, and colloids (Minnesota Rural Water Association, 2001).

Nanofiltration utilises membranes of very small pore sizes. Due to their very small pore size, they require a higher operating pressure compared to micro and ultrafiltration (Table 1) (Gurol, 2011). Nanofiltration systems provide a higher level of treatment than the micro and ultrafiltration systems and have the added capability of removing dissolved organic contaminants as they can remove nearly all cysts, bacteria, viruses, and humic materials. They provide excellent protection from disinfectant by-products (DBPs) formation if the disinfectant residual is added after the membrane filtration step (Gurol, 2014). Reverse osmosis (RO) is effective for the removal of virtually all inorganic contaminants from water (Minnesota Rural Water Association, 2001). It is also known to effectively remove radium, natural organic substances, pesticides, cysts, bacteria, and viruses. Reverse osmosis is particularly effective when used in series with multiple units (Allende & Monaghan, 2015). An important advantage of membrane filtration is their ability to act directly on microorganisms, leaving the properties and composition of water virtually intact (Biryukov *et al.*, 2005). Pollutants are also removed from the water phase without degradation. By physically removing the pathogens, membrane filtration can significantly reduce the use of chemicals (Minnesota Rural Water Association, 2001). This prevents the formation of degradation products and possible disinfection by-products (DBPs) that occur when chemicals are used (Gurol, 2014). Be that as it may, membrane filtration only partially solves the problem of pollutants in water since the resulting concentrate needs to be treated (Guchi, 2015). This technology is only effective at controlling pathogens when combined in a series of multi-stage filters and in combination with other treatments that have other modes of actions. Most of the time, membrane technologies are used in combination with chemical or biological processes (Minnesota Rural Water Association, 2001). Another limiting factor is membrane fouling, which results in the deterioration of membrane operation, the need for fouling control consequently leads to the increase of water treatment cost (Molelekwa *et al.*, 2014). Also, due to the pre-treatment and the high pressure required for membranes, the energy costs of reverse osmosis (RO) and nanofiltration (NF) are high (Biryukov *et al.*, 2005).

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Table 1 Operational and technical differences observed between membrane processes used for water disinfection (Minnesota Rural Water Association, 2001; Gurol, 2011).

Description	Microfiltration	Ultrafiltration	Nanofiltration	Reverse osmosis
Molecular weight	> 1000 000 Da	10 000 - 100 000 Da	1000 – 100000 Da	
Operating pressure	100 - 400 kPa	200 - 700 kPa	600 - 1000 kPa	
Pore size	0.1 - 10 µm	0.002 - 0.1 µm	0.0005-0.002 µm	<0.0005 µm
Retained particles	> 300 000	1 000 – 300 000	> 150	< 350

Biochar filtration

Introduction, mode of action, advantages and disadvantages

The production and use of biochar are part of the modern agenda to retain pollutants, recycle wastes, and to offset some greenhouse gas emissions (Brantley *et al.*, 2015). Biochar filtration has, therefore, over the past few years gained interest in the water industry as a low-cost water treatment method (Ahmad *et al.*, 2017; Gwenzi, 2017). Biochar is produced by the pyrolysis of carbon-rich biological material at high temperatures in the absence of oxygen (Dalahmeh, 2016). This pyrolysis results in a porous material with beneficial properties that serve as an adsorbent and biofilm carrier for water treatment (Dalahmeh, 2016). The capacity of biochar filters to remove pollutants, however, differs between materials and depends on characteristics such as porosity, specific surface area and reactivity, adsorption capacity and ability to promote biofilm development (Ahmad *et al.*, 2017). Pine biochar is a type of a biochar that is derived from pine wood. It is generally made from the composition of 65-75% carbohydrate polymers and oligomers, 18-35% lignin and 4-10% of low molecular weight compounds (Fidel, 2015). Pine biochar filtration has been shown to be effective in the removal of both of organic or inorganic pollutants including heavy metals (Dalahmeh, 2016; Ahmad *et al.*, 2017; Van Rooyen, 2018). It has also shown great

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potential in improving the physico-chemical parameters of water (Brantley *et al.*, 2015; Van Rooyen, 2018). A disadvantage of this method, however, is that the pyrolysis process can be costly considering the price of electricity and the indirect impact on the environment when producing electricity and charring organic matter (Brantley *et al.*, 2015).

Photochemical treatment methods

This method involves a reaction in which a chemical dimer is formed from the absorption of light by the DNA or RNA of a microorganism causing cell death (Bolton & Linden, 2003).

Introduction to ultraviolet light

Ultraviolet (UV) light is found in sunlight and is part of the electromagnetic spectrum. It is shorter wavelenght than visible light but longer than X-rays (Edstrom, 2011; Turtoi, 2013). The UV spectrum ranges from 100 to 400 nm and is divided into distinct spectral regions including, UV-C (100-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm) (Figure 3) (Clarke & Bettin, 2006). Ultraviolet-C is considered the germicidal range because it generates short UV waves in the region of 254 nm (the most effective spectral region for germicidal purposes) (Clarke & Bettin, 2006; Mounaouer & Abdennaceur, 2012). Ultraviolet light irradiation has emerged as a leading water treatment method and is one of the most effective disinfection methods for bacteria, viruses, and parasites in water (Oguma *et al.*, 2001; IWG, 2002). The light inactivates microorganisms in the following efficiency order: Protozoa (*Cryptosporidium* and *Giardia*), bacteria, bacterial spores, viruses and bacteriophages (Parke & Fisher, 2012; Turtoi, 2013).

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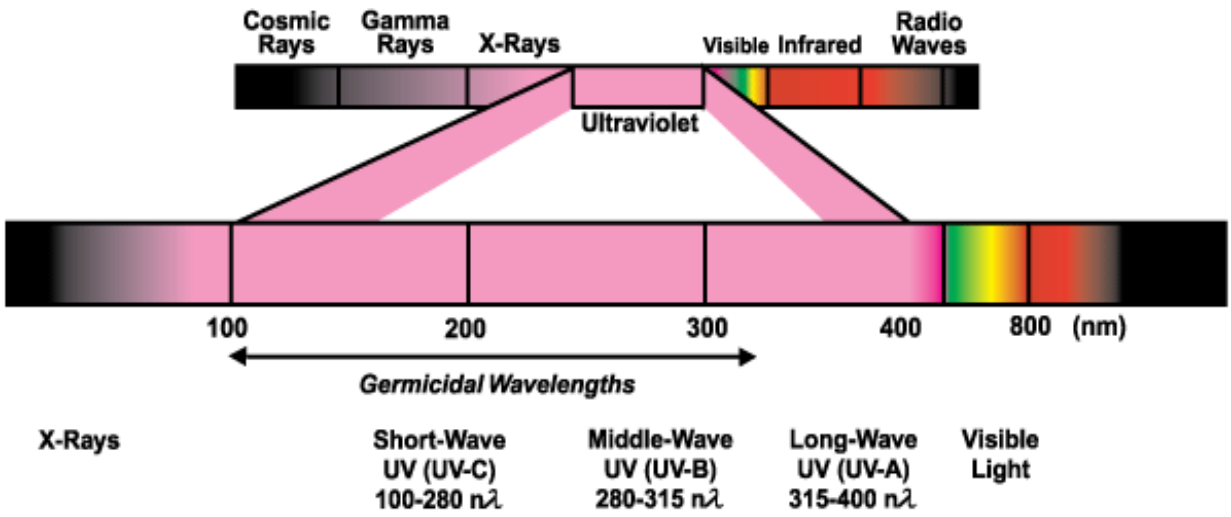

Figure 3 Schematic representation of UV in the electromagnetic spectrum (IWG, 2002).

Types of UV designs used

Generally, the UV disinfection system is made up of a reactor, mercury arc lamps and a control box (Turtoi, 2013). The UV lamp contains an inert gas (e.g. argon) and a small amount of liquid mercury and is commonly used to emit UV light for water disinfection (Clarke & Bettin, 2006). The source of radiation is either a low pressure (LP) or medium pressure (MP) mercury arc lamp with low or high intensities respectively (Turtoi, 2013). These terms are based on the vapour pressure of mercury when the lamps are operating. The LP lamps operate at mercury vapour pressures of 2×10^{-3} – 2×10^{-5} pounds per square inch (psi) while, MP lamps operate at a much higher mercury vapour pressures of 2 – 200 psi at a higher intensity (Clarke & Bettin, 2006). Medium-pressure lamps also operate at a much higher temperature range of 600-900° C as compared to LP lamps that operate at temperatures of 40 – 200° C (Clarke & Bettin, 2006). Low-pressure lamps have been commonly used in the past because they efficiently produce UV rays in the range lethal to microbes (Morita *et al.*, 2002). Low-pressure UV lamps emit monochromatic light at a wavelength of 254 nm, which is the peak of germicidal effectiveness for most microbes (Quek & Hu, 2008). About 50% of the LP energy input is converted to UV rays having a wavelength of 254 nm (IWG, 2002; Oguma *et al.*, 2002), yielding a major wavelength output of about 85% (Freese & Nozaic, 1999). It is for this reason, that LP lamps are often referred to as ‘‘germicidal’’ lamps (Bolton & Linden, 2003).

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Medium-pressure UV lamps have been used because of their much higher intensities. They are mostly used for large facilities (Freese & Nozaic, 1999). Medium-pressure UV lamps emit over a broad range of wavelengths, including germicidal wavelengths from 200 to 300 nm, which affects the DNA and RNA plus other biological molecules such as proteins and enzymes, enabling greater inactivation impact (Bolton & Linden, 2003). The MP lamps disinfect faster and have greater penetration capability due to the high intensity. As the radiation from MP UV lamps is polychromatic, only about 12% of the energy input is converted to the germicidal UV-C region (Quek & Hu, 2008).

Principle of UV

The principal cause of inactivation by UV irradiation involves the absorption of UV light by the DNA or RNA of a microorganism (Bolton & Linden, 2003). Ultraviolet light penetrates the cell membranes to impact directly on the DNA molecules, which absorb energy from the radiation (Quek & Hu, 2008). This absorption creates damage in the DNA by altering nucleotide base pairing, thus causing a formation of photoproducts in DNA (Morita *et al.*, 2002). The most important of these photoproducts is the formation of cyclobutane pyrimidine dimer (CPDs) between adjacent pyrimidine molecules on the same strand of DNA (Figure 4) (Zimmer & Slawson, 2002). The CPD photolyase or 6 – 4PPs (6 – 4 photolyase) are also formed on about 25% of CPDs (Gayán *et al.*, 2013b). The dimers inhibit the formation of new DNA or RNA chains in the process of cell replication (mitosis) thus interrupting both the transcription and replication of the DNA (Bolton & Linden, 2003; Nebot Sanz *et al.*, 2007). If the DNA is not repaired, replication does not take place which results in cell death (Gayán *et al.*, 2013b).

Microbial death may not happen instantly, but the scrambling of the genetic material in the nucleus prevents reproduction, rendering it non-viable and harmless to humans (Nebot Sanz *et al.*, 2007). The amount of energy required to produce this effect in a given organism is referred to as the lethal dosage. The degree of inactivation by ultraviolet irradiation is directly related to the UV dose applied (International Water Guard, 2002).

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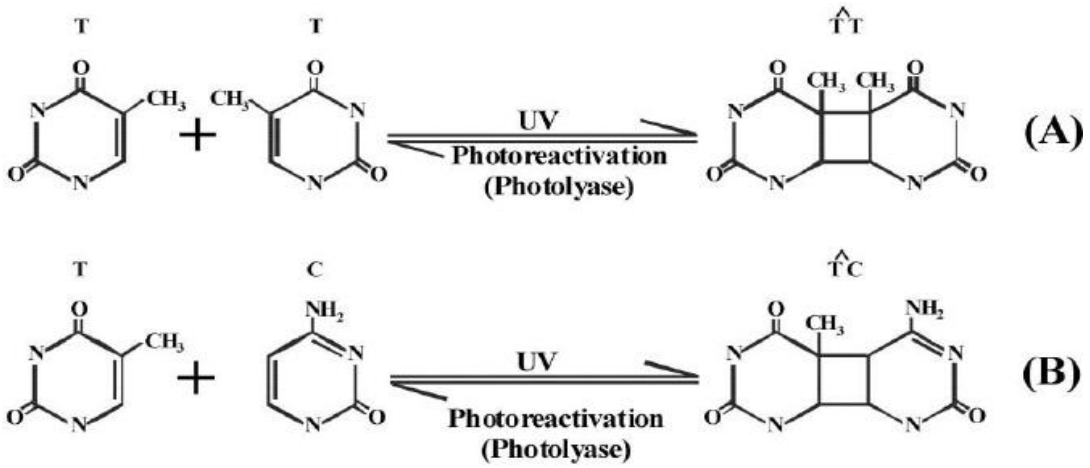

Figure 4 Schematic representation of the formation of DNA lesion, cyclobutane pyrimidine by UV irradiation (Sinha & Häder, 2002).

Advantages of UV irradiation

Over the past years, UV irradiation has gained popularity and interest in water disinfection due to many advantages it has over other water disinfection methods (Khan *et al.*, 2015). The major advantage of UV irradiation as a primary disinfection process came after the discovery of its high efficacy against many pathogenic microorganisms (Oguma *et al.*, 2001). When sufficient dosage is applied, UV light is fatal to all microorganisms known to inhabit water (IWG, 2002). It was demonstrated that UV radiation is very effective against *Cryptosporidium* and *Giardia,* the two most problematic waterborne pathogens (Heijnen & Medema, 2006).

Another notable advantage of UV irradiation is the fact that it does not produce hazardous disinfection by-products (DBPs) which results from the reaction of chemicals with organic matter in water (Oguma *et al.*, 2002). This is because, UV irradiation is a photo process, which does not require any chemical input, the only thing that is added to the water is the energy (Mounaouer & Abdennaceur, 2012). Therefore, there is no danger of chemical overdose (Turtoi, 2013). The UV process also eliminates the need for specialised hazardous materials knowledge or training since no dangerous chemicals need to be used, handled, transported or stored (Turtoi, 2013; Khan *et al.*, 2015). These make it a safer option for the operator and the community than chemicals, which can be toxic if released (Morita *et al.*, 2002). Another advantage of UV disinfection over other disinfectants is that inactivation does not depend on water temperature and pH. Generally, the temperature and pH of water

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does not have a significant effect on UV disinfection efficacy. Temperature can affect the activity of repair enzymes and nucleic acid configuration, which may require a very slight increase in UV dose. The pH affects the activity of repair enzymes and nucleic acid configuration, but pH within a cell is relatively constant and does not vary with water pH (Clarke & Bettin, 2006). Moreover, UV disinfection has extremely shorter contact time (ranging from seconds to a few minutes with LP lamps) compared to other disinfectants such as filtration (Turtoi, 2013). The short contact times coupled with the small space required by the UV reactors and easy maintenance have contributed to its rising popularity as an alternative disinfection process (Teksoy *et al.*, 2011). Another technological advantage of using UV disinfection is that it lacks sensitivity to temperature variations. Series of batch inactivation studies with bacteria, yeasts, and viruses supported this evidence as it demonstrated that the effect of temperature on UV inactivation was very small (Nebot Sanz *et al.*, 2007). Also, unlike chemical disinfectants such as ozone, the biological stability of the water is not affected by UV lamps (Heijnen & Medema, 2006). Ultraviolet irradiation thus solves the environmental and safety problems (Mounaouer & Abdennaceur, 2012), making it a highly economical and viable technology (Quek & Hu, 2008).

Disadvantages of UV

Despite all the positive aspects, UV has drawbacks. The major disadvantage of UV disinfection is the ability of some microorganisms to repair DNA lesions by mechanisms such as photoreactivation and dark repair (Morita *et al.*, 2002; Oguma *et al.*, 2002). These mechanisms reverse the damage caused by UV irradiation and allow inactivated microorganisms to be reactivated (Quek & Hu, 2008). Another common negative comment about the use of UV irradiation in water treatment is the lack of a residual disinfection agent (IWG, 2002). Ultraviolet light does not show any residual activity in water after disinfection, this might create room for possible re-contamination if care is not taken (Freese & Nozaic, 1999). Turbidity and total suspended solids (TSS) in the water can furthermore, affect the UV disinfection efficacy (Turtoi, 2013). UV rays should hit the microorganism directly. In the case where the water contains suspended particles, they shield the microorganism from the UV rays and reduce the disinfection efficiency (Biryukov *et al.*, 2005). Ultraviolet disinfection with low-pressure lamps is not as effective for secondary effluent with TSS levels above 30 $mg.L^{-1}$ (Turtoi, 2013). Moreover, some stubborn viruses, spores, and cysts may not be effectively

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inactivated by low dosage, thus requiring high UV doses (Turtoi, 2013). Although there is no technical limitation on the size of a UV plant, more units being added to larger plants tend to increase the operating costs at larger facilities (Freese & Nozaic, 1999).

Photoreactivation and dark repair

Photoreactivation is a phenomenon in which UV-inactivated microorganisms recover activity through the repair of lesions in the DNA by photolyase enzymes in the presence of visible light (Oguma *et al.*, 2001). This issue has received a lot of attention because it greatly affects UV disinfection efficiency (Guo *et al.*, 2009). Photoreactivation is probably one of the simplest and oldest repair systems consisting of a single enzyme: photolyase (Sinha & Häder, 2002). The ability of microorganisms to perform photo repair depends on whether they have the enzyme photolyase. During UV treatment, the UV light causes damage to the DNA of the microorganism where dimers of pyrimidine are created. These, dimers prevent the bacteria from duplicating (Locas *et al.*, 2008). In order to remove these DNA lesions formed by UV, the photolyase enzyme actually binds to CPDs (CPD photolyase) or 6 – 4PPs (6 – 4 photolyase) and reverses the damage using the energy of light (Sinha & Häder, 2002). Once the damaged sites in the DNA are repaired, the microorganism will then be able to carry out reproduction and re-contaminate the disinfected water (Quek & Hu, 2008).

The reaction takes place in less than a millisecond. Consequently, the limiting step of the whole reactivation process is the formation of the pre-dimer complex Photoreactivation is also influenced by the intensity and duration of visible light exposure, UV dose and type of UV lamps used to disinfect the water (Locas *et al.*, 2008). An extended period of exposure to photo reactivating light would enable the release of pre-dimers, which would then be available to form new complexes (Salcedo *et al.*, 2007). These may occur in microorganisms of UV-exposed water, since microorganisms in surface water are normally exposed to sunlight, including near-UV light (Morita *et al.*, 2002). Photoreactivation will be different after disinfection by low or medium-pressure UV lamps (Oguma *et al.*, 2002; Zimmer & Slawson, 2002; Locas *et al.*, 2008). It has been reported that MP UV lamps have an advantage over LP lamps for water disinfection in terms of the photoreactivation of bacteria (Guo *et al.*, 2009). The broad range of wavelengths applied by MP lamps reduces photoreactivation (Bolton & Linden, 2003). It was shown that MP UV lamps produce a broad, ‘‘polychromatic" spectrum of UV wavelengths that inflict irreparable damage not only

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on cellular DNA but on other molecules (Nebot Sanz *et al.*, 2007). On the other hand, LP lamps emit a single wavelength peak which only affects DNA (Nebot Sanz *et al.*, 2007). It is for this reason that, microorganism reactivation is more difficult with MP lamps. A study conducted by Zimmer and Slawson (2002), clearly showed photo repair of *E. coli* following exposure to LP UV irradiation but no repair was detected after exposure to MP UV source at the initial doses of less than 10 $mJ.cm^{-2}$ examined. Their study showed that after exposure to LP UV irradiation, *E. coli* repair under photo reactivating light increased rapidly, reaching maximum levels at about 2 to 3 hours after UV irradiation before levelling off. Some studies have also noted that if reactivation is observed in a microorganism, the extent of reactivation is often inversely related to the UV dose applied (Oguma *et al.*, 2002; Nebot Sanz *et al.*, 2007; Salcedo *et al.*, 2007; Locas *et al.*, 2008).

The repair generally tends to be higher after low UV doses (Guo *et al.*, 2009). A study by Zimmer and Slawson (2002), also showed a decrease in DNA repair at higher doses, as the time needed for repair increased with an increase in UV dose. This is because higher UV doses induce greater damage to the DNA. It takes longer therefore, to repair the damage, since there are only approximately twenty photolyase enzymes in each *E. coli* organism and each enzyme can repair only approximately five dimers per minute. According to Zimmer and Slawson's results, although photo repair was observed after each dose (5, 8 and 10 $mJ.cm^{-2}$) of LP irradiation, the levels of repair did not reach the initial concentration of *E. coli* before UV exposure. It was thus concluded that complete repair does not really occur. Guo *et al* (2009) however, dismissed the advantage of MP lamps over LP lamps saying, photoreactivation does not occur when high UV doses are employed no matter which type of lamp was used. In their study, photo repair was not detected when the UV dose was 15 $mJ.cm^{-2}$ for either LP or MP lamps. From this, it was concluded that any type of UV lamp can be used for water treatment if a high germicidal UV dose is used (Guo *et al.*, 2009). In dark repair, UV-inactivated microorganisms repair the damaged DNA in the absence of light. Dark repair may occur in UV-exposed water after it is distributed by water supply systems (Morita *et al.*, 2002). This repair process involves the action of more than a dozen proteins that coordinate the removal of DNA damage (Zimmer & Slawson, 2002). The ability for dark repair is known to differ greatly from species to species (Oguma *et al.*, 2001).

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Effects of water quality parameters on UV efficacy

While UV irradiation provides effective disinfection against many microorganisms, it is important to note that the efficiency and reliability of UV disinfection are greatly dependent on the intensity of UV light to which the microorganisms are exposed (Teksoy *et al.*, 2011). In order to cause damage, the UV light must pass through the water (Avery *et al.*, 2016). Ultraviolet transmittance (UVT) which measures the UV light's ability to penetrate through a water sample unobstructed, determines the amount of light that reaches the microorganism to cause cell death (Clarke & Bettin, 2006). Ultraviolet light does not penetrate solid materials (Avery *et al.*, 2016). Ultraviolet transmittance is, therefore, reduced if the water being disinfected is contaminated. Certain contaminants present in water reduce the light that passes through the water (Edstrom, 2005). These contaminants are not limited to dissolved particles, but can also include suspended particles such as soils and organic matter (Edstrom, 2005).

In general, suspended and dissolved particles in water scatter UV light making the water appear turbid (cloudy or murky). Dissolved substances, notably natural organic matter and especially organic matter which gives colour to water, absorb UV light (Avery *et al.*, 2016). Humic substances such as humic and fulvic acids are also known to absorb UV light (Teksoy *et al.*, 2011). Suspended particles in water reduce the effectiveness of disinfection treatments by absorbing UV light and shielding microbes from UV light thereby increasing microbial survival (Mounaouer & Abdennaceur, 2012). The more UV light is absorbed by such substances, the lower the intensity reaching the microorganisms (Avery *et al.*, 2016). Microorganisms associated with substances are thus not destroyed (Turtoi, 2013).

Water with high levels of turbidity has been shown to decrease the action of disinfectants (Turtoi, 2013). A study done by Wobma (2004), showed that turbidity levels up to 5 NTU did not affect UV efficacy. However, UVT and disinfection efficacy were reduced at 13 NTU. Low turbidity is no guarantee that water is free from pathogenic microorganisms (Clarke & Bettin, 2006). A minimum requirement, therefore, for a potential UV disinfection installation, is that the water is clear and not cloudy (Avery *et al.*, 2016). Total suspended solids should ideally be less than 20 $mg.L^{-1}$. Higher amounts of suspended solids can interfere with water disinfection treatments (Parke & Fisher, 2012). Dissolved substances such as organic matter, iron,

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manganese and hardness may deposit over time on the sleeve which separates the UV lamp from the water, a process known as fouling. Fouling reduces the intensity of UV light entering the water. Table 2 shows the maximum levels of certain contaminants that are allowable for effective UV treatment.

Table 2 Recommended maximum contaminant levels in water entering a UV treatment device (Wagenet *et al.*, 2015).

Contaminants	**Recommendations**
Turbidity	≤ 5 NTU*
Suspended solids	≤ 20 $mg.L^{-1}$
Colour	None
Iron	0.3 $mg.L^{-1}$
Manganese	0.05 $mg.L^{-1}$
pH	6.5-9.5
UVT	>75 %

2.8. SUMMARY

It is an undeniable fact that water is an important part of human life. Many researchers have highlighted its importance by describing the roles it plays in domestic, agricultural and industrial products (Blignaut & Van Heerden, 2009). It is also well known by many that water, although very important, has become a very scarce resource. Developments caused by population growth have not only decreased the availability of water but also contaminated the available water (Paulse *et al.*, 2012). Contaminated surface water poses a risk to human health in cases where the water is used for irrigation of fresh produce that is intended to be consumed raw (Uyttendaele *et al.*, 2015). Contaminated irrigation water is one way by which pathogenic microorganisms can reach the produce (Zimmer & Slawson, 2002). Countries worldwide have reported problems associated with pathogens in fresh produce (FAO/WHO, 2008). For this reason, treating river water before it is used for irrigation purposes is very important in reducing the danger of disease outbreaks following the consumption of such products. Ultraviolet irradiation has emerged as a leading water treatment method because of several advantages, such as high disinfection efficiency of most viruses, bacteria and protozoa, no unidentified toxic DBPs and safe operation (Guo *et al.*, 2009). Despite all these advantages, many researchers have reported on the light-induced damage

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repair of DNA that occurs in many microorganisms after UV irradiation (Morita *et al.*, 2002; Oguma *et al.*, 2002). Therefore, the current study aims at investigating the effect of river water quality on LP UV disinfection efficacy and the phenomenon of photoreactivation.

2.9. CONCLUSION

As demonstrated in many reviews, rivers in the Western Cape especially those in Stellenbosch are reasons for concern (Paulse *et al.*, 2009, 2012; Britz *et al.*, 2013). These rivers are polluted with faecal matter and carry high microbial loads which often exceed the limit that is set by WHO and DWAF (FDA /WHO, 2008; Britz *et al.*, 2013). Polluted river water presents great risks when used for irrigation of fresh produce (Pachepsky *et al.*, 2011). In fact, contaminated irrigation water has been scrutinised as a vehicle for foodborne microorganisms and has been implicated in many disease outbreaks (Uyttendaele *et al.*, 2015). Water disinfection is therefore of primary importance to inactivate pathogenic organisms capable of causing foodborne outbreaks (Turtoi, 2013). Ultraviolet irradiation has potential as a water disinfection method (Guo *et al.*, 2009).

There are, however, certain water quality related issues that affect the performance of UV. It is a well-established scientific fact that the efficiency and reliability of UV disinfection are greatly dependent on the quality of water (Morita *et al.*, 2002). The presence of UV absorbing substances in the water may reduce the intensity of UV light passing through water by sheltering organisms from UV radiation and scattering UV light (Avery *et al.*, 2016). Substances that can decrease UVT include natural organics, phenolic compounds, some metals and anions such as iron and manganese (Avery *et al.*, 2016). In domestic-scale UV treatments, UV efficacy is often tested on waters with high UV transmittance (usually >75% UVT) (Avery *et al.*, 2016). Due to contamination from the environment, UVT of river waters is often below <75%. The potential efficacy of UV water treatment is thus likely to be compromised (Avery *et al.*, 2016). River water also contains a variety of microorganisms, some of which may be pathogens. Generic *E. coli* has been enumerated from rivers in Stellenbosch, however, knowledge is limited about the presence of important produce-related pathogens such as STEC in these rivers. Studies have shown a correlation between generic *E.coli* and the presence of pathogens (Tallon *et al.*, 2005). In many laboratory

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studies, effective UV disinfection has been observed when reference *E.coli* strains are used to test UV disinfection efficacy in saline solutions. Practically, however, river water may contain naturally occurring environmental strains some of which may be resistant to UV because they have been shown to be better adapted to adverse environmental conditions (Oguma *et al.*, 2001). Due to constant exposure to UV light from the sun in the environment, some microorganisms develop resistance to UV irradiation. Furthermore, Some of these microorganisms also possess mechanisms to repair UV-damaged DNA (Oguma *et al.*, 2001). Photoreactivation which greatly affects the efficacy of UV disinfection reverses UV induced damage (Oguma *et al.*, 2001). The ability to perform photoreactivation differs between different microorganisms, and even between different strains of the same species (Hijnen *et al*., 2006; Gayán et al., 2014). Due to differences in both intrinsic and extrinsic parameters, some microorganisms are easier to reactivate than others. Most strains of *E. coli* are known to be capable of photoreactivation (Oguma *et al.*, 2001). It is, therefore, crucial to quantitatively evaluate the effects of photoreactivation, so as to achieve an appropriate UV disinfection dosage (Morita *et al.*, 2002).

Regarding dosage, different UV dosage is required by different microorganisms to effectively inactivate microorganisms. While some microorganisms require very high UV doses, others only require as low as 5 $mJ.cm^{-2}$. It is therefore important to extensively study the minimum effective dose required to achieve the desired microbiological goal (Bolton & Linden, 2003). Thus, it is relevant to investigate the correct dosages required for disinfection of a variety of waterborne microorganisms and the phenomenon of photoreactivation by bacteria after exposure to UV light (Sigge *et al*., 2016). In addition to investigating the phenomenon of photoreactivation, it is also important to evaluate the effects of water quality parameters on UV disinfection efficacy using river water.

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CHAPTER 3

INVESTIGATING LOW-PRESSURE (LP) ULTRAVIOLET (UV) DISINFECTION EFFICACY OF SELECTED SHIGA-TOXIN *E. COLI* STRAINS IN WATER

ABSTRACT

This study investigated the disinfection efficacy of UV-C irradiation against five *Escherichia coli* strains which included three Shiga-toxin *Escherichia coli* (STEC) strains. An American Type Culture Collection (ATCC 35218) reference strain and four *E. coli* strains (F11.2, STEC EH, STEC DP and STEC 210) in sterile Ringer's solution were exposed to five different UV doses (20, 30, 40, 50 & 60 mJ.cm^{-2}). Population numbers decreased as the UV dose increased for all the *E. coli* strains tested. The desired 3 log CFU.mL^{-1} reduction was achieved, even at the lowest dose of 20 mJ.cm^{-2}. Variations in UV sensitivity among *E. coli* strains were, however, observed. Log reductions ranged between 3.6 - 4.4 log for the lowest dose (20 mJ.cm^{-2}) and between 5.3 - 6.2 log for the highest dose (60 mJ.cm^{-2}). STEC EH was the most UV sensitive strain with the highest log reductions of (4.4, 5.3, 5.4, 5.8, 6.2 log CFU.mL^{-1}) at all five UV doses (20, 30, 40, 50 and 60 mJ.cm^{-2}), respectively. An environmental *E. coli* (F11.2) and two STEC isolates (STEC 210 and STEC DP) were more UV resistant than the ATCC 35218 reference strain. Amongst these strains, STEC DP and F11.2 were the most UV resistant strains with STEC DP being the most resistant strain at doses 30 and 40 mJ.cm^{-2} with F11.2 being more resistant at doses 50 and 60 mJ.cm^{-2}. Based on these results, the influence of water quality was investigated by inoculating the environmental STEC strain (STEC 210) into both autoclaved river water and Ringer's solution and UV irradiating it at three different UV doses (20, 40, 60 and mJ.cm^{-2}). Again, results showed a direct relationship between an increase in UV dose and an increase in log reduction. Results also showed that log reductions did not differ significantly. It was concluded that, water quality parameters did not influence LP UV disinfection efficacy, provided that the correct dose was applied.

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INTRODUCTION

Agricultural irrigation uses about 62% of the accessible fresh water in South Africa (Britz *et al.*, 2013). Despite its significant role in food production, the availability of water for irrigational purposes is decreasing due to many factors (Ijabadeniyi & Buys, 2012). Observations are that the microbiological quality of available water is also, decreasing rapidly (Pachepsky *et al.*, 2011; Paulse *et al.*, 2012). Surface waters, the most predominant water source of agricultural irrigation can be contaminated with faecal coliforms (Pachepsky *et al.*, 2011; Paulse *et al.*, 2012; Uyttendaele *et al.*, 2015). Irrigation water has thus become a focal point in produce safety because it is well recognised as a potential avenue for produce contamination with faecal coliforms (*E. coli*) (EPA, 2013). Although most strains of the *E. coli* group are non-pathogenic, certain strains contain virulent genes that may cause various human-related illnesses (Ndlovu *et al.*, 2015).

Shiga toxin-producing *E. coli* (STEC), a class of enteric pathogens, remains a major foodborne pathogen of concern across the globe (Verhaegen *et al.*, 2016). The bacterium, which can be transmitted to humans through consumption of contaminated raw or undercooked beef, raw milk, fresh produce and contaminated water (Verhaegen *et al.*, 2016), has been implicated in many foodborne outbreaks and is believed to be widespread in rivers (Quiros *et al.*, 2015). The presence of STEC in irrigation water sources is, therefore, of major concern due to the high risk of food- and waterborne outbreaks (Ram *et al.*, 2011). This emphasises the need to treat water before it is used for irrigation of fresh produce. Ultraviolet irradiation, which directly impairs the intracellular functions of microbial cells (Owoseni *et al.*, 2017), has been shown to effectively inactivate waterborne pathogens when a sufficient dose is applied (Zimmer & Slawson, 2002; Gayán *et al.*, 2013). A problem with UV is that UV sensitivity varies between different strains of the same organism as well as, between different species (Hijnen et al., 2006; Gayán et al., 2014). As such, it is crucial to determine how certain microorganisms respond to UV treatment in the water matrix in which the organism is found or is inoculated (Bolton & Linden, 2003). *Escherichia. coli* reference strains are commonly used in laboratory studies to assess the efficiencies of treatment methods. This is, however, not an accurate method to test for efficiency of treatment method because, naturally occurring environmental strains may be better adapted to adverse environmental conditions that enable them to withstand higher UV doses

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(Guo *et al.*, 2009; Olivier, 2015). This study used a combination of both reference and environmental *E. coli* strains, including STEC, to determine the efficacy of UV disinfection in water. The objective of this study was to evaluate the UV irradiation sensitivity of various STEC strains so as to determine the optimal germicidal UV dose required to inactivate STEC. The potential influence of river water quality parameters on UV disinfection efficacy was also investigated.

MATERIALS AND METHODS

General materials and methods

Escherichia coli strains

An *E. coli* reference strain (ATCC 35218), a non-pathogenic environmental strain (F11.2) and three STEC strains were used to investigate UV disinfection efficacy (Table 1). All strains were preserved at -80°C in 40% ($v.v^{-1}$) glycerol. To confirm the STEC isolates, the DuPont™ BAX System real-time PCR assay (Hygiena) that detects STEC (*stx* and *eae*) genes was used according to the procedure described in the BAX System User Guide. The strains were individually inoculated in buffered peptone water (BPW) (Oxoid, South Africa) and incubated at 37°C overnight. Following this, 20 µL of this enrichment broth was transferred to 200 µL prepared BAX lysis reagent in cluster tubes. Lysis was performed by heating the tubes for 20 min at 37°C and 10 min at 95° C, then cooling tubes at 4°C for 5 min. About 30 µL of the lysate was then transferred to PCR tubes which were subsequently loaded into the BAX System Q7 instrument. A full process was run and analysed using software version 3.2 for standard assays.

Table 1 The five *E. coli* strains used in laboratory-scale disinfection experiments

***E. coli* strain**	**Source**	**STEC BAX confirmation**
ATCC 35218	Reference strain	
***E. coli* F11.2**	Environmental (Plankenburg River)	
STEC EH	Clinical strain	Positive
STEC DP	Clinical strain	Positive
STEC 210	Environmental (Game meat)	Positive

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Inoculum preparation

Inoculum preparation was carried out according to Quek & Hu (2008), with modifications to suit this study. About 200 µL of conserved *E. coli* culture was transferred into 5 mL tryptic soya broth (TSB) (Oxoid, South Africa) and incubated at 37ºC for 24 h. Following incubation, a loop full of *E. coli* suspension was streaked on Levine's Eosin Methylene-Blue Agar (L-EMB) agar (Oxoid, South Africa) which was also incubated at 37ºC for 24 h to confirm strain purity (*E. coli* colonies appear as metallic green colonies on L-EMB agar). A single *E. coli* colony was then picked from the L-EMB plate using a sterile loop and inoculated into 5 mL TSB (Oxoid, South Africa) and incubated for 24 h at 37ºC. The cells were collected by centrifugation (1 500 × g, 12 min) (TJ-25; Beckman, South Africa), rinsed and subsequently suspended in sterile Ringer's solution (quarter strength) or autoclaved river water to yield a cell density equivalent to a 0.5 McFarland standard (BioMèrieux, South Africa), with an optical density (OD) value of 0.117 and an approximate microorganism concentration of 10^{-7} CFU.mL^{-1}

River water sampling site and method

River water was sampled from the Eerste, Krom and Plankenburg Rivers (Stellenbosch) according to the procedure described by the South African National Standards (SANS) 5667-6 method (SANS, 2006). Sampling at specific sites was done using a sampling stick containing a sterile 1L beaker that was submerged in the river. The water was then transferred to a sterile 2L reagent bottle that was kept cool during transport to the laboratory where it was analysed.

Figure 1 Photographs of three rivers sampled: *a*= Eerste River, *b*= Krom River and *c*= Plankenburg River

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Ultraviolet disinfection

Ultraviolet disinfection tests were carried out according to the standard collimated beam test protocol described by Bolton & Linden (2003) with modification according to Guo *et al* (2009). A bench-scale collimated device (Berson, The Netherlands) (Figure 2) which emitted monochromatic light at 254 nm was used. The UV instrument used an Amalgam Low-pressure mercury vapour lamp (UV-Technik, Germany) with a power output of 40 W and an arc length of 25 cm. Ultraviolet light intensity at the sample surface was determined before each treatment using an ILT1400 radiometer (International Light Technologies, USA) coupled with an XRL140T254 detector (International Light Technologies, USA). For UV disinfection, a 250 mL beaker containing 25 mL of the sample was placed on the horizontal surface of a magnetic stirrer below the bottom of the collimator, and gently stirred with the aid of a magnetic stirrer bar for a pre-determined time of UV exposure to yield desired doses of 20, 30, 40, 50 and 60 mJ.cm^{-2}. The time of exposure to deliver a specific UV dose was calculated according to Hallmich & Gehr (2010).

$$I\text{avg, }\lambda\ (mW.\ cm^{-2}) = I0\lambda\ [\frac{1-e^{d\ln(\mathrm{UVT}(\lambda))}}{-d\ln(\mathrm{UVT}(\lambda))}]\ [1]$$

$$Desired\ dose\ (mJ.\ cm^{-2}) = Average\ intensity\ (mW.\ cm^{-2}) \times Exposure\ time\ (s)\ [2]$$

In the equation above, *I*(avg,λ) is the average intensity of UV light over the sample depth, *d*; UVT(λ) refers to the UV transmission at wavelength, λ, determined using an optical path length of 1 cm; I0(λ) is the intensity of UV light measured at the surface of the sample.

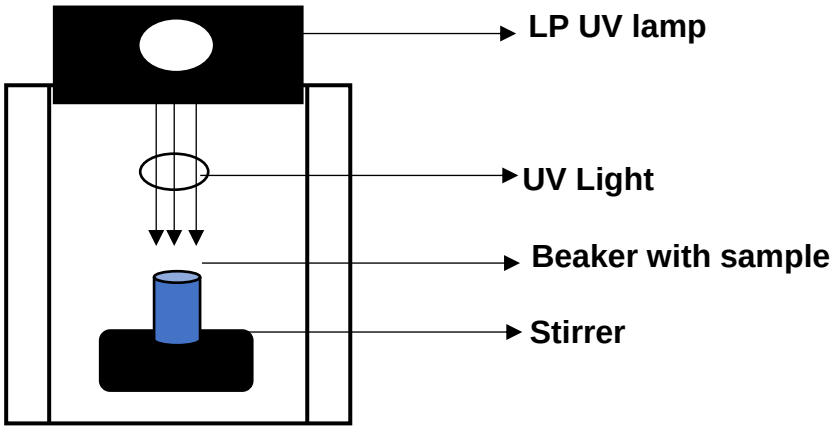

Figure 2 Representation of UV collimated beam device used in the UV experiments

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Microbiological analysis of samples

Microbial analysis of samples was performed using standard pour plate method. Following UV irradiation, irradiated samples were serially diluted according to the South African National Standards (SANS) method 6887-1 (SANS, 1999) in 9 mL Ringer's solution. Dilutions (10^{0} – 10^{-8}) were prepared before (control) and after all specific UV disinfection treatments. Violet Red Bile Glucose Agar (VRBG) (Merck, South Africa) was used to prepare duplicate pour plates which were incubated at 37°C for 24 h. *Escherichia. coli* counts were determined according to the SANS method 4832 (SANS, 2007a). *Escherichia. coli* appears in the form of red colonies on VRBG agar. The colony counts between 25 – 250 per plate were recorded.

Physico-chemical analysis of river water

Chemical Oxygen Demand (COD)

The COD, an indicative measure of the amount of oxygen that can be consumed by oxidation reactions in a measured solution, was determined photometrically using the Spectro quant Nova 60 COD cell test (Merck Millipore, South Africa) measuring in the range between 10 – 150 mg $O_2.L^{-1}$. Three millilitres of each water sample was added to a COD test vial, containing all the required reagents, and vortexed before analysis. The samples were digested for 2 h at 150°C in a thermal reactor (Hach, USA). Following sample digestion, the reaction cells were cooled to room temperature before measuring the COD value of the solution.

Ultraviolet transmission percentage (UVT%)

The UVT% of river water samples were determined using a sense T254 UV Transmission % Photometer (Berson, Netherland) according to the instructions provided by the manufacturer. Distilled water was used for the calibration of the instrument and represented UVT % of 100%.

Turbidity

A portable Orion AQ3010 Turbidity Meter (Thermo Scientific, USA) was used to determine the turbidity, measured as Nephelometric Turbidity Units (NTU), of river water samples. The instrument was calibrated before analysis using known turbidity values. Analysis of the river water was performed in duplicate.

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Electrical Conductivity (EC)

A portable HI 8733 conductivity meter (Hanna Instruments, USA) was used to quantify the number of dissolved salts in river water samples. Calibration of the instrument and the adjustment of measuring units were performed according to the instructions provided by the manufacturer.

Alkalinity

Alkalinity, total suspended solids (TSS) and volatile suspended solids (VSS) were carried out according to instructions by Standard Methods (APHA, 2005). To test for alkalinity, 0.1 N H_2SO_4 was titrated into 20 mL of sample to reach a pH of 4.3. The titrated volume was used to calculate alkalinity. TSS and VSS were gravimetrically determined at 105 ^oC and 550 ^oC respectively.

pH

A portable pH meter (WTW, Germany) was used, according to the manufactures instructions to determine the pH of river water samples. The instrument was calibrated before use. The analysis was performed in duplicates.

Statistical analysis

Statistical analysis was done with 13.3 Statistica software (StatSoft, USA) using a two-way analysis of variance (ANOVA). Significant results were identified by means of a 95% confidence interval, where F- probability was significant at ($P<0.05$).

Research study design

Part 1: Evaluating the efficacy of UV disinfection on STEC strains

In order to investigate the LP UV disinfection efficacy of selected *E. coli* (including STEC) strains, several laboratory-scale experiments were performed. The UV sensitivity of each *E. coli* strain was investigated in a similar manner. Strain suspensions were individually prepared for five *E. coli* isolates (ATCC 35218, F11.2, STEC EH, STEC DP and STEC 210) (Table 1), using sterile Ringer's solution. Each suspension was exposed to five different UV doses (20, 30, 40, 50 and 60 $mJ.cm^{-2}$). To determine the log reduction of *E. coli* strain at the tested UV doses, enumeration of each of the strains was done before and after UV disinfection treatment. This was done by serially diluting the treated and untreated suspensions, and duplicate pour

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plates were prepared using VRBG agar (Merck, South Africa). The plates were incubated at 37°C for 24 h. All experimental procedures were performed in triplicate.

Part 2: Effects of water quality on UV disinfection potential

For the second part of the study, the influence of water quality (measured in terms of organic matter content (COD), UV transmission percentage (UVT %), turbidity, suspended solids content (TSS), volatile suspended solids (VSS) and conductivity) was investigated. This was done by using autoclaved river water from different sources as diluents. Water was collected from three different rivers (Eerste, Krom and Plankenburg). Each river water sample was individually investigated. For each treatment, a selected STEC strain (STEC 210) was inoculated into both Ringers solution and autoclaved river water. The inoculum was exposed to three different UV doses of 20, 40 and 60 $mJ.cm^{-2}$. Enumeration procedures were performed as described for UV disinfection studies in Part 1. Log reductions of STEC in Ringer's solution were compared to that of STEC in autoclaved river water samples.

RESULTS AND DISCUSSION

Part 1: Evaluating the efficacy of LP UV disinfection on STEC strains

Studies have demonstrated the effectiveness of UV irradiation against many microorganisms (Morita *et al.*, 2002; Zimmer and Slawson, 2002; Gayán *et al.*, 2013; Jones *et al.*, 2014). Information regarding UV resistance of significant water and foodborne bacteria is, however, limited (Gayán *et al.*, 2014). This study was, therefore, conducted to establish the inactivation response of five *E. coli* strains to varying levels of UV irradiation from a LP UV lamp. Colony counts of each *E. coli* strains before and after UV treatment (at different doses) are presented in Table 2. An inverse relationship between population size and UV dose was observed. Similar to previous findings (Yan *et al.*, 2017), of UV sensitivity of *E. coli* strains, the population size of the strains in this study, decreased with every increase in UV dose. It was also observed that, the decrease in numbers with every increase in UV was less than a log for all strains (Table 2).

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Table 2 Recorded colony count (log CFU. mL^{-1}) of each *E. coli* strain before and after treatment with five UV doses

		UV Dose (mJ.cm^{-2})				
Strain	**Before UV**	**20**	**30**	**40**	**50**	**60**
F11.2	7.74±0.59	3.94±0.17	3.66±0.26	3.45±0.31	3.30±0.40	2.54±0.35
ATCC 35218	7.85±0.86	4.29±0.31	3.49±0.06	2.83±0.12	2.28±0.12	1.89±0.06
STEC EH	7.73±0.72	3.33±0.30	2.77±0.53	2.45±0.31	2.17±0.56	1.47±0.12
STEC 210	7.69±0.92	4.26±0.26	3.96±0.46	3.24±0.62	2.68±0.60	2.07±0.42
STEC DP	7.49±0.64	3.23±0.17	3.59±0.12	3.33±0.25	2.84±0.47	2.14±0.40

Results also indicated a substantial difference in UV sensitivity among the different *E. coli* strains tested (Figure 3). The UV sensitivity of an organism is described by the degree of log reduction observed after irradiation. A UV sensitive strain would have a higher log reduction value than more UV resistant strains. In this study, UV treatment at all five doses (20, 30, 40, 50 and 60 mJ.cm^{-2}) resulted in more than a three-log reduction. Log reductions ranged between 3.9 - 5.5 log, 3.8 - 5.3 log, 3.7- 6.0 log, 4.4 - 6.2 log, and 3.7- 5.8 log for STEC DP, F 11.2, STEC EH, ATCC 35218 and STEC 210, respectively (Figure 3). In terms of indicators, a three log reduction implies that, should *E. coli* levels in water be around 10^5 – 10^6 CFUs per 100 mL^{-1}, the treatment might be able to reduce the counts to meet the agricultural irrigation guideline limit of 1 000 *E. coli* per 100 mL^{-1} (Britz *et al.*, 2013; Olivier, 2015). This is important, especially for rivers like the Plankenburg that often carry very high *E. coli* counts (Paulse *et al.*, 2009; Lamprecht *et al.*, 2014).

To achieve a log reduction of 5.3 for the *E. coli* F11.2 strain, a UV dose of 60 mJ.cm^{-2} was required, while the same log reduction was achieved with only a UV dose of 30 mJ.cm^{-2} for the STEC EH strain. This clearly indicates variation in UV sensitivity of different strains within the same species. Increased resistance to UV was in this order; STEC EH < ATCC 35218 < STEC 210 < STEC DP < *E. coli* F11.2. Even though the orders of UV sensitivity of the *E. coli* strains were not consistent at each UV dose, STEC EH was undoubtedly the most UV sensitive strain, which showed the highest log reductions at all doses tested (Figure 3). The ATCC 35218 strain was the second most sensitive to UV and showed greater overall UV sensitivity than the two STEC (STEC DP and STEC 210) and environmental *E. coli* (F11.2) isolates (Figure 3).

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Variation in UV sensitivity of different organisms or even different strains of the same species have been reported before (Gayán *et al.*, 2014; Olivier, 2015; Yan *et al.*, 2017). Sommer *et al.* (2000) showed great variation in UV sensitivity between eight *E. coli* strains in water. In their study, a UV dose of 12 mJ.cm^{-2} was enough to achieve 6-log reduction for the most susceptible strain O157:H7 (CCUG 29199), while a dose of 125 mJ.cm^{-2} was needed for the most resistant strain. Yan *et al.*, (2017) also demonstrated variation in UV sensitivity between pathogenic and non-pathogenic *E. coli* strains. It was found that, pathogenic *E. coli* strains were significantly more sensitive than the non-pathogenic strains at a UV dose of 51 mJ.cm^{-2}.

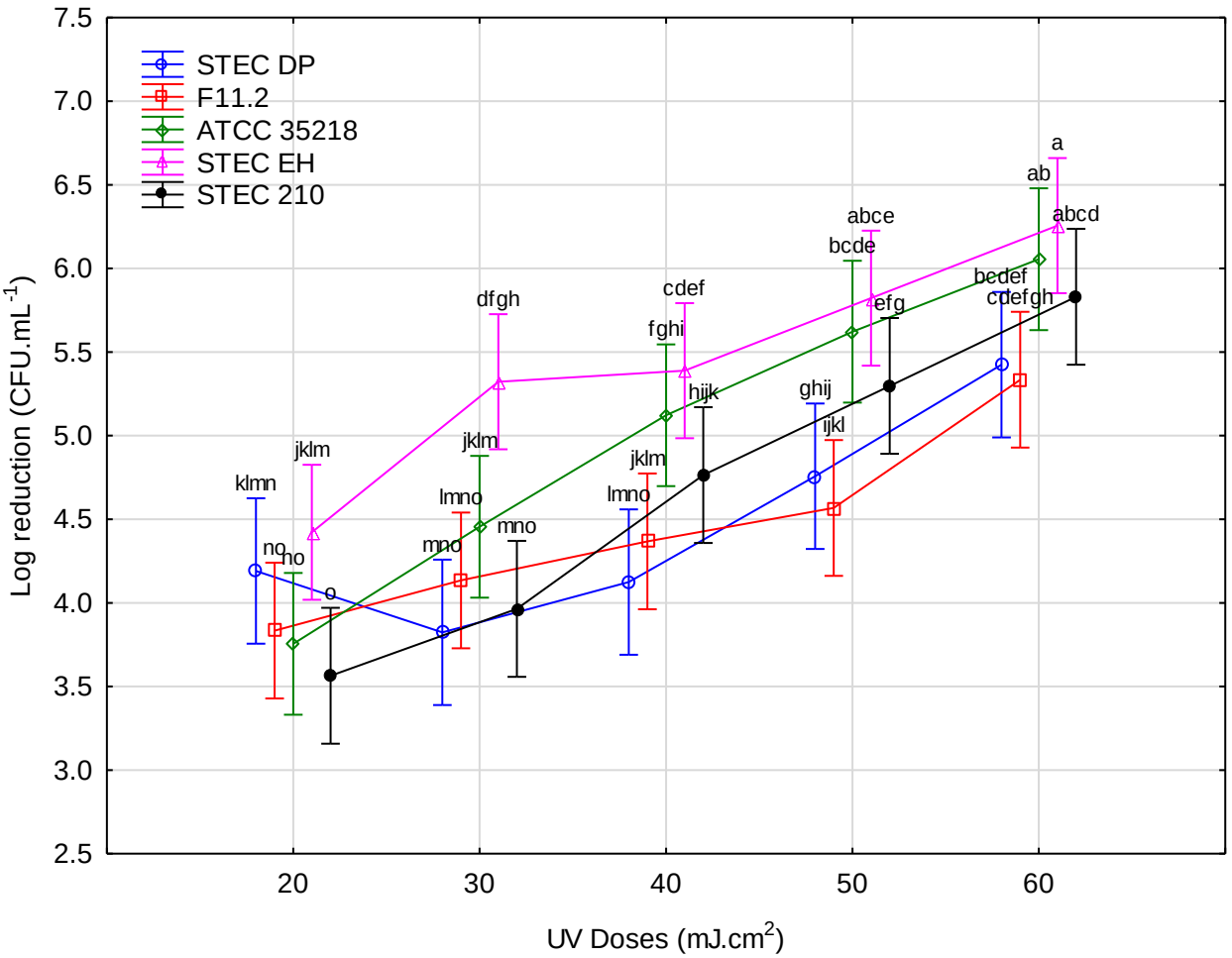

Figure 3 Disinfection efficiency of UV doses (20, 30, 40, 50 & 60 mJ.cm^{-2}) on five *E. coli* strains in Ringer's solution. Error bars were calculated based on the standard deviation at a confidence interval of 0.95.

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While differences in UV sensitivity were observed among the *E. coli* strains, it appeared in this study, that some differences were not statistically significant (P> 0.05) at certain UV doses. At UV dose 20 mJ.cm^{-2}, STEC 210 was significantly more resistant than STEC DP and ATCC 35218 (Figure 3). At dose 30 mJ.cm^{-2}, STEC DP, STEC 210, *E. coli* F11.2 and ATCC 35218, were significantly more resistant than STEC EH. At dose 40 mJ.cm^{-2}, STEC DP was significantly more resistant than STEC 210, STEC EH and ATCC 35218. At dose 50 mJ.cm^{-2}, *E. coli* F 11.2 was significantly more resistant than STEC EH, ATCC 35217 and STEC 210. At dose 60 mJ.cm^{-2}, *E. coli* F 11.2 and STEC DP were significantly more resistant than STEC EH and ATCC 35218. Overall, STEC EH and ATCC 35218 were significantly more sensitive than at least one other strain at all UV doses.

Similar to these findings, Sigge *et al.* (2016), from their study also concluded that environmental strains were more UV resistant than reference strains in water. Furthermore, when Mofidi *et al.* (2002) compared the UV sensitivity of an *E.coli* reference strain to that of a clinical strain in sterile distilled water, they found that the reference STEC (ATCC 23229) strain was more UV sensitive than the clinical isolate (O157: H7) at UV doses 5 and 11 mJ.cm^{-2}.

According to Gayán *et al.* (2014), variation in sensitivity may be due to differences in process parameters, microbial characteristics and product parameters. UV sensitivity is a microbial characteristic of each microorganism which may depend on both intrinsic and extrinsic factors (Sommer *et al.*, 2000). With regards to intrinsic factors, UV sensitivity varies widely depending on the microorganism, species, and strain. The resistance of environmental strains may be attributed to the fact that, environmental strains are better adapted to harsh environmental conditions. Microorganisms may constantly be exposed to stress conditions in their environment which can make them resistant to treatment (Gayán *et al.*, 2014).

Part 2: Effects of water quality on UV disinfection potential

To further investigate the effect of water quality on UV disinfection efficacy, an environmental STEC strain (STEC 210) was inoculated into both autoclaved river water and Ringer's solution. Strain choice was based on the results of Part 1 as well as the initial source of the isolate. STEC 210 was an environmental strain and also had one of the highest resistances to UV at low doses (20 and 30 mJ.cm^{-2}) (Figure 3). It was thus considered to be a good representative strain of the five strains in this

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study. Physico-chemical analysis of the river water was done prior to UV disinfection. Results presented in Table 3 show variation in physico-chemical parameters among the three different rivers sampled. Comparatively, water from the Plankenburg River showed the highest alkalinity, COD, conductivity, turbidity, TSS and VSS values. These results were positively correlated with the very low UVT% value represented by the Plankenburg River (Table 3). High COD value may have been a result of increased organic and inorganic pollutants in the river (Rajiv *et al.*, 2012). This was demonstrated by high levels of conductivity, turbidity, TSS and VSS (Table 3). High levels of turbidity, particulate matter, and natural organic matter absorb more UV light, resulting in a decreased UVT%. With regards to UV disinfection, low UVT% have been shown to reduce UV disinfection effectiveness by absorbing UV light and shielding microbes from UV light (Morita *et al.*, 2002; Gayán *et al.*, 2014). In terms of UV dosage, low UVT% translates to longer UV exposure time.

Table 3 Water quality parameter of Ringers solution and water from different rivers.

Properties	**Irrigation standard**	**Ringer's solution**	**River water**		
Source			**Eerste**	**Krom**	**Plankenburg**
Alkalinity (mgCaCO3.L^{-1})	30 - 130	10	200	225	475
COD (mg.L^{-1})	10	<10	33	38	45
Conductivity (mS.m^{-1})	≤ 40	0.07	15	23	62
pH	6.5-9.5	6.70	7.20	7.12	7.4
Turbidity (NTUs)	≤ 5 NTU*	6.30	7.56	6.49	166
TSS (mg.L^{-1})	≤ 20	0.004	10	12	146
UVT%	>75 %	77	44	46	14.4
VSS (mg.L^{-1})	-	0.002	21	35	400

Contrary to other findings regarding the effects of water quality on UV disinfection efficacy (Gayán *et al.*, 2014; Khan *et al.*, 2015; Sigge *et al.*, 2016), water quality in this study did not affect the UV disinfection efficacy of specific UV doses. Results shown in Figure 4 do not indicate significant differences ($P>0.05$) in bacterial log reductions between autoclaved river water and Ringer's solution at all three respective doses (20, 40 and 60 mJ.cm^{-2}). Log reductions of STEC 210 in Ringer's and River water were

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4.18 and 4.01 log, at dose 20 mJ.cm^{-2}, 4.55 and 4.44 log at dose 40 mJ.cm^{-2} and 5.12 and 5.60 log at dose 60 mJ.cm^{-2}, respectively. The restraining impact of pollutants on UV effectiveness at a specific dose was thus not a significant factor in this study. Water quality did however, affect the UV exposure time required to achieve the desired UV doses (20, 40 and 60 mJ.cm^{-2}). As shown in Table 4, the UV exposure times of autoclaved river water were longer than that of Ringer's solution at all UV doses. This was done to compensate for the low UVT% of the river water samples.To effectively achieve a UV dose of 40 mJ.cm^{-2} for both Ringer's solution and river water, the Plankenburg river water had to be exposed to UV for 19.50 minutes as compared to Ringer's solution that was exposed for only 9.08 minutes (Table 4). In agreement with this, Khan *et al.*, (2015) stated that, particles in highly turbid water scatter the UV rays which reduces the UVT% , hence, longer exposure times are required for UV disinfection of water.

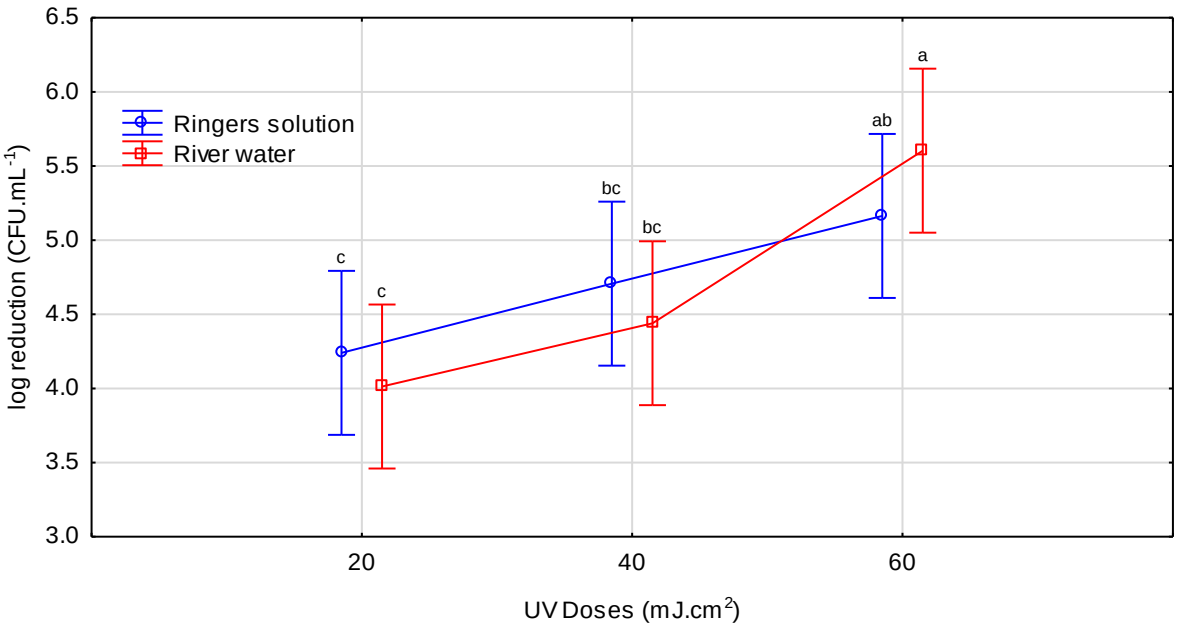

Figure 4 Log reduction of STEC 210 in Ringer's solution and River water at UV doses (20, 40, & 60 mJ.cm^{-2}). Error bars were calculated based on standard deviation at a confidence interval of 0.95

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Table 4 UVT (%) and calculated UV exposure times (min) of Ringer's solution and River water

Diluent:	Ringer's solution	Rivers		
		Plankenburg	Krom	Eerste
UVT (%)	77	14.4	46	44
UV doses (mJ.cm^{-2})	**Exposure time (min:sec)**			
20	4:09	9:07	6:20	6:30
40	9:08	19:50	12:46	12:59
60	14:07	29:20	18:59	19:01

CONCLUSION

As stated, a desired three log reduction in colony counts was achieved at all UV doses. These results suggest that UV irradiation has a great potential for inactivating waterborne microorganisms such as *E. coli* and STEC. It was also observed that increased UV doses resulted in increased log reductions. Therefore, if greater microbial reduction is required, higher UV doses should be applied by increasing the UV exposure time for the sample or by improving the physico-chemical parameters that negatively affect UVT%. Similarly, if a small dose is applied, it can be expected to result in lower log reductions. The log reduction results also clearly showed variation in sensitivity among *E. coli* strains at all UV doses. Observations where that, two environmental strains (STEC 210 and F11.2) and a clinical STEC strain (STEC DP) were generally more UV resistant than the ATCC 35218 strain. One strain can therefore not be considered representative of a whole microbial species in UV disinfection studies. Strain choice for treatment-optimisation studies needs to be made carefully. With regards to water quality influence, physico-chemical parameters did compromise UV disinfection efficiency in that longer UV exposure times were required for highly turbid water to achieve the same amount of inactivation.

This study therefore, highlights the fact that, the limitation of water quality can be overcome by increasing the exposure time to ensure the correct dose. Furthermore, improvement of water quality by pre-treatment would also make higher log reductions more easily achievable. Knowing the UVT% of a sample, therefore, is essential for UV disinfection applications to ensure the target dose is applied. On the other hand, the

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influence of water quality was investigated using only one environmental STEC strain (STEC 210). The question remains whether a mixed microbial population will show the same degree of inhibition and needs further investigation. This also demonstrates that the use of a single environmental STEC strain to determine UV disinfection efficacy may underestimate the resistance of STEC strains occurring in mixed populations in natural environments.

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CHAPTER 4

INVESTIGATING THE LOW-PRESSURE (LP) ULTRAVIOLET (UV) DISINFECTION EFFICACY OF RIVER WATER

ABSTRACT

The disinfection efficacy of ultraviolet (UV) irradiation was tested on river (Eerste, Krom and Plankenburg) waters with varying contaminant levels. The Eerste River was found to be fit for irrigational purposes with *Escherichia coli* (*E. coli*) levels and physico-chemical parameters falling within the recommended irrigation water guidelines levels. The Krom River met the guidelines set by the South African Department of Water Affairs and Forestry (DWAF), in terms of physico-chemical and *E. coli* counts. Further microbial analysis of the river water, however, revealed the sporadic presence of STEC and ESBL producing *Enterobacteriaceae* in the water which raised questions of whether or not this river water is safe for irrigation of fresh produce. The Plankenburg River carried the highest *E. coli* loads. The water was also found to harbour STEC and ESBL producing *Enterobacteriaceae*. From these results, it was concluded that the disinfection of river water prior to irrigation is necessary. River water samples from all rivers were then exposed to UV irradiation at two UV doses (40 and 60 mJ.cm^{-2}). *Enterobacteriaceae*, total coliforms and *E. coli* were enumerated and STEC detected before and after UV disinfection was conducted. Total reduction of *Enterobacteriaceae*, total coliforms and *E. coli* were achieved for the Eerste and Krom Rivers at both UV doses (40 and 60 mJ.cm^{-2}). For the Plankenburg River, a desired 3 log reduction was achieved at both UV doses (40 and 60 mJ.cm^{-2}), with the higher UV dose (60 mJ.cm^{-2}) resulting in greater reduction. With regards to STEC, a UV dose of 40 mJ.cm^{-2} was unable to inactivate STEC in the Plankenburg River samples. Considering the impact of water quality, high suspended solids could have contributed to the reduced UVT%, which resulted in increased UV exposure time. Microorganism repair of all microbial population tested was observed following UV irradiation at both UV doses (40 and 60 mJ.cm^{-2}). However, the higher dose (60 mJ.cm^{-2}) resulted in less recovery.

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INTRODUCTION

In South Africa, river water is used for the irrigation of fresh produce, often without treatment (Ndlovu *et al.*, 2015). Rivers, however, carry high levels of *E. coli*, originating from the indiscriminate disposal of sewerage, industrial waste and human activities (Paulse *et al.*, 2009; Pachepsky *et al.*, 2011; Ndlovu *et al.*, 2015; Olivier, 2015). The presence *E. coli* in irrigation water is a major concern, due to the emergence of pathogenic *E. coli* such as Shiga-toxin producing *E.coli* (STEC) (Lascowski *et al.*, 2013; Rawway *et al.*, 2016). Shiga-toxin producing *E.coli* are foodborne pathogens that can cause serious human illness, such as haemolytic uremic syndrome (HUS) through the production of one or more Shiga-like toxins genes (encoded by *stx1* and *stx2* and their variants) (Heijnen & Medema, 2006; Lascowski *et al.*, 2013). The occurrence of STEC in surface waters has been reported in many countries (Ram *et al.*, 2011; Ennis *et al.*, 2012; Vital *et al.*, 2018). In South Africa the *stx* gene was detected in river water samples collected from the Berg River (Paarl, South Africa) (Ndlovu *et al.*, 2015).

Apart from causing illnesses, another problem with *E. coli* is that it can easily acquire resistance to antibiotics consumed by humans and animals (Zarfel *et al.*, 2017). This includes resistance caused by extended-spectrum-beta-lactamases (ESBLs). Extended-spectrum-beta-lactamases are enzymes that confer resistance to nearly all beta-lactam antibiotics including, penicillin and broad-spectrum (first, second, and third-generation) cephalosporins (Kittinger *et al.*, 2016). Resistance is the result of CTX-M, TEM and SHV-type enzymes, and genes are often encoded on plasmids, which can easily be transferred between bacteria (Rupp & Fey, 2003; Guyomard-Rabenirina *et al.*, 2017). Antibiotic resistance limits effective therapy in the case of possible bacterial infection (Rupp & Fey, 2003; Poulou *et al.*, 2014), and can be spread through the water environment (Zarfel *et al.*, 2017). Extended-spectrum-beta-lactamases genes have previously been isolated from river and lake water samples in other countries (Rupp & Fey, 2003; Shehani & Lui, 2013; Zurfluh *et al.*, 2013). In South Africa, antibiotic resistance has been observed among river water isolates (Olaniran *et al.*, 2009; Romanis, 2013; Lamprecht *et al.*, 2014). Hence, it is important to treat river water before it is used for irrigation, in order to prevent the possible transfer of antibiotic-resistant pathogens to fresh produce during irrigation (Lamprecht *et al.*, 2014).

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In this regard, ultraviolet (UV) irradiation is widely recognised as a technology available to rid water of microorganisms (Mofidi *et al.*, 2002; Mounaouer & Abdennaceur, 2012; Jones *et al.*, 2014). This nonthermal/nonchemical technology uses UV light to effectively inactivate microorganisms (Morita *et al.*, 2002). As with any method, UV irradiation is not flawless. Factors such as water quality and DNA repair have been shown to compromise the effectiveness of the technology (Sommer *et al.*, 2000; Quek & Hu, 2008). With regard to water quality, certain particulate substances in water can influence UV disinfection as a result of the light being scattered and/or blocked, instead of being absorbed (Teksoy *et al.*, 2011). The presence of DNA repair mechanisms reverses the UV induced damage, allowing inactivated microorganisms to be reactivated (Quek & Hu, 2008). Photo repair (photoreactivation) and dark repair (occur in the absence of light) are two main repair pathways used to reverse UV-induced DNA damage (Zimmer & Slawson, 2002). In addition, re-growth of microorganisms resulting from extra nutrients following UV disinfection may also result in recovery (Olivier, 2015). Dark repair has been reported to be significantly less influential (Guo *et al.*, 2009; Olivier, 2015). In this regard, photo repair, and re-growth of the microorganisms are, therefore, of great concern in water treatment because they allow microorganisms to re-contaminate the disinfected water (Oguma *et al.*, 2002). Studies have reported on the repair of microorganisms (especially *E. coli*) after exposure to UV irradiation (Oguma *et al.*, 2002; Zimmer & Slawson, 2002; Jones *et al.*, 2014). The aim of this study was to, therefore, investigate LP UV disinfection potential against river water. The impact of water quality parameters and the recovery potential of microorganisms were also investigated.

MATERIALS AND METHODS

General materials and methods

Sampling design and sample collection

Water samples were collected from three different rivers (Eerste, Krom and Plankenburg) in Stellenbosch (Figure 1). One sampling point was selected for each river and each river was sampled three times. A total of nine samples were collected between May and June 2018. Samples were collected using a sampling rod containing a sterile beaker, that was submerged in the river. The water was then transferred to a

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sterilised 2L sampling bottle that was transported to the laboratory for analysis. All samples were analysed within 6 h of collection.

Figure 1 Sampling sites at three rivers sampled: *a*= Eerste River, *b*= Krom River and *c*= Plankenburg River

Physico-chemical analysis of river water

Physico-chemical analysis were done on all of untreated river water samples. Electrical conductivity, pH and turbidity were measured using a portable HI 8733 conductivity meter (Hanna Instruments, USA), pH meter (WTW, Germany), and a portable Orion AQ3010 Turbidity Meter (Thermo Scientific, USA), respectively. To determine alkalinity, 0.1 N H_2SO_4 was titrated into 20 mL of sample to reach a pH of 4.3. COD value was measured in mg O_2. L^{-1}, using a spectra quant Nova 60 COD cell test (Merck Millipore, South Africa) measuring in the range between 10 – 150 mg O_2. L^{-1}. total suspended solids (TSS) and volatile suspended solids (VSS) were gravimetrically determined at 105^{o}C and 550^{o}C, respectively according to Standard Methods (APHA, 2005). A total dissolved solids (TDS)-3 meter (HM Digital) that measures the electrical conductivity of water or the total amount of mobile charged ions found in the water was used to estimate the TDS in water. Ultraviolet transmission percentage (UVT%) was measured using a Sense T254 UV Transmission % Photometer (Berson, Netherland) according to the manufacturer‘s instructions. Distilled water was used for calibration and represented UVT of 100%.

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Microbiological analysis of river water

Standard plating methods and membrane filtration (MF) methods were used for microbial analysis of the river water samples. In brief, serial dilutions (10^0 – 10^{-6}) were prepared according to the South African National Standards (SANS) method 6887-1 (SANS, 1999) in 90 and 9 mL of Ringer's solution for membrane filtration and standard plating methods respectively. For MF, 100 mL of each dilution was filtered through sterile cellulose nitrate membrane filters with pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa) as specified by the U.S. Environmental Protection Agency (USEPA) method 1604 (USEPA, 2002). The membrane filter was then placed onto Chromogenic Coliform Agar (Oxoid, South Africa) and incubated at 37ºC for 24 h. Total coliforms and *E. coli* were observed as salmon to red and dark blue to violet colonies, respectively.

For standard plating, serial dilutions (10^0 – 10^{-6}) were prepared in 9 mL Ringer's solution before (control) and after UV disinfection. Aliquots of 1 mL of each dilution were then plated out on duplicate Violet Red Bile Glucose (VRBG) agar (Merck, South Africa) plates for the enumeration of *Enterobacteriacea*e and on Chromogenic coliform agar (Oxoid, South Africa) for the simultaneous enumeration of total coliforms and *E. coli*. All plates were inverted and incubated at 37°C for 24 h.

Detection and isolation of Extended-Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae

Isolation of ESBL producing colonies

In order to test for the presence of ESBL producing *Enterobacteriacea*e in untreated river water, 100 mL of river water was filtered through sterile cellulose nitrate membrane filters with pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa) as described by USEPA (2002). The filters were transferred to 20 mL of buffered peptone water (BPW) and incubated at 37°C for 2 h. Following incubation, 1 mL of the sample was transferred into 9 mL of EE broth (Merck, South Africa) and further incubated for 24 h at 37°C. A loopful of the suspension was then streaked on ChromID Brilliance ESBL agar (bioMérieux, South Africa) and again incubated at 37°C for 24 h. The ChromID Brilliance ESBL screening agar contains a mixture of antibiotics, including cefpodoxime, two chromogenic substrates, and one natural

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substrate, to enable direct species identification (Poulou *et al.*, 2014). Growth on the plates was considered as ESBL presumptive positive colonies and was sub-cultured into Tryptone soya broth (TSB) (Merck, South Africa) and stored at – 80ºC in 40% glycerol until further analysis. The colours of the colonies were recorded according to the colour chart provided by the manufacturer (*E. coli*: pink/burgundy; *Klebsiella/Enterobacter/Serratia*: Blue/green; *Proteus*: light to dark brown) (Figure 2).

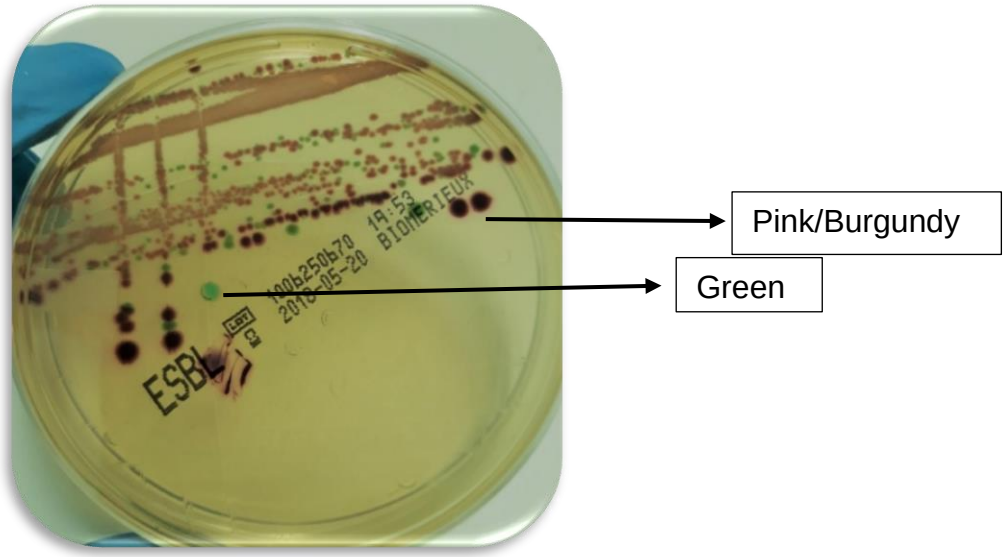

Figure 2 Image of presumptive positive ESBL producing colonies with different colours on a ChromID Brilliance ESBL agar plate

Identification of ESBL producing Enterobacteriaceae

Isolates from different coloured colonies were considered potential ESBL producers and were further identified using Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF) mass spectrometry (Bruker Daltonik GmbH, Germany). One microlitre of prepared isolate supernatant was pipetted onto a MALDI target plate (Bruker Daltonics GmbH, Germany) and allowed to dry at room temperature. Each sample was then covered with 1.0 µL of a saturated solution of alpha-cyano-4-hydroxycinnamic acid matrix (Bruker Daltonics GmbH, Germany) and allowed to dry at room temperature. Two spots were systematically prepared for each isolate. The Bruker Daltonics MALDI-TOF Biotyper identification results are colour-coded as follows: highly probable species identification= dark green, secure genus identification, probable species identification = light green, probable genus identification = yellow

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and not reliable identification = red. Confidence levels between 70.0% and 99.9% were considered correct identification at the genus and species levels (Amelita Lombard, 2018, Agricultural Sciences Building, University of Pretoria, Personal communication).

Antimicrobial susceptibility test (ESBL confirmation 1)

The isolates identified as *Enterobacteriaceae* with MALDI-TOF analysis were subjected to antimicrobial susceptibility tests using the disc diffusion technique according to the European Committee on Antimicrobial susceptibility testing (EUCAST) guidelines (EUCAST, 2012). Colonies of each isolate were suspended in sterile TSB to obtain a turbidity level equal to 0.5 McFarland standard (BioMérieux, South Africa). The test was performed by inoculating duplicate Mueller-Hinton agar (MHA) (Davis diagnostics, South Africa) plates with 0.1 mL isolate suspension. After this, the following discs were applied to the inoculated MHA plates using a disc dispenser (Oxoid, South Africa): Cefotaxime (CTX) (30 mg), Ceftazidime (CAZ) (30 mg) and Cefepime (CPM) (30 mg) alone and in combination with Clavulanic acid (CA) (10 mg) (Davis diagnostics, South Africa). The plates were incubated at 37°C for 24 h. The tests were considered positive for Group 1 ESBL producers when an increase in the growth-inhibitory zone around either the CTX or the CAZ disc with CA was 5 mm or greater than the diameter around the disc containing CTX or the CAZ alone (Poulou *et al.*, 2014). The tests were considered positive for Group 2 ESBL producers when an increase in the growth-inhibitory zone around the CPM disc with CA was 5 mm or greater than the diameter around the disc containing CPM alone (Poulou *et al.*, 2014)

In addition to the ESBL production confirmation test, resistance to three other antibiotics from different classes of antimicrobials was also tested. These antibiotic classes included penicillins (Ampicillin 30 µg), amphenicols (Chloramphenicol 30 µg) and tetracyclines (Tetracycline 30 µg) (Davis diagnostics, South Africa). The selection of the above antibiotics was based on a previous study on resistant *E. coli* isolated from similar water sources (Romanis, 2013), and the World Health Organisation (WHO)'s list of critically important antimicrobials for human health (WHO, 2016). Zones of inhibition were measured with a calliper and interpreted as resistant, intermediate or sensitive using the interpretative chart of the zone sizes of the Kirby – Bauer sensitivity test method (Poulou *et al*., 2014) (Table 1). The tests were performed in duplicate. For quality control, *E. coli* ATCC 25922 reference strain was used as a

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negative control because it is susceptible to all the antibiotics included in this study (EUCAST, 2012). The *E. coli* ATCC 35218 reference strain was included as a positive control since it carries the β-lactamase TEM-1 gene (Romanis, 2013), and was also resistant to all the antibiotics used in this study.

Table 1 Inhibition zone criteria for interpreting the antibiotic resistance of *Enterobacteriaceae* (EUCAST, 2012)

	Diameter of inhibition zones		
	Sensitive ≥ (mm)	Intermediate (mm)	Resistant ≤ (mm)
Ampicillin 10 µg	15	14-16	14
Chloramphenicol 30 µg	18	13-17	12
Tetracycline 30 µg	15	12-14	11

Detection of ESBL Genes (ESBL confirmation 2)

Multiplex polymerase chain reaction (PCR) was used to test for the presence of beta-lactamase (*bla*) TEM, SHV and the CTX-M genes in all the MALDI-TOF identified *Enterobacteriaceae* isolates, using the primer pairs presented in Table 2. The DNA template preparation for PCR was performed in this manner: Pure colonies of an overnight nutrient agar (Merck, South Africa) of the test organism were transferred to 100 µL sterile nuclease-free water (VWR, Internationals) in a microcentrifuge tube. This was then boiled in a thermocycler for 13 min and after which it was centrifuged at 14 000 x g. The supernatant which contained the nucleic acid material was transferred to a sterile PCR tube and stored at – 80°C until PCR testing. Each PCR reaction mixture of 12.5 µL consisted of 1.25 µL primer mix, 6.25 µL Kapa2GFast multiplex mix, 3.75 µL RNase-free water and 1.25 µL DNA template. polymerase chain reaction amplification was performed in a thermal cycler (Vacutec, South Africa) under the following conditions: heat denaturation at 95°C for 15 min, followed by 30 cycles of denaturation at 94°C for 30 s; annealing at 60°C for 30 s and extension at 72°C for 2 min. This was followed by a final extension step at 72°C for 10 min and 4°C hold. The PCR products were analysed by gel electrophoresis in a 1% agarose gel (SeaKem, Switzerland) containing 1 µg. mL^{-1} EZ-Vision DNA dye (Sigma, Germany) in 0.5% TAE buffer. The PCR bands were visualised on a UV-transilluminator (Vacutec, South

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Africa). A 100-base pair (bp) DNA ladder, a positive control (isolate containing all three genes) and a negative control were included in each run.

Table 2 Primer pairs used for amplification of the TEM, SHV and CTX-M ESBL types

Primer	Sequence (5' - 3')	Base pair	Reference
bla-SHV.SE	ATGCGTTATATTCGCCTGTG	747	Paterson *et*
bla-SHV.AS	TGCTTTGTTATTCGGGCCAA		*al*, 2003
TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	Monstein *et*
TEM-165.AS	ACGCTCACCGGCTCCAGATTTAT		*al*, 2007
CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGG	593	Boyd *et al*,
CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCG G		2004

Detection of STEC in water samples

Determination of BAX system sensitivity

The sensitivity of the DuPont™ BAX System Real-Time PCR assay (Hygiena) to detect STEC (*stx* and *eae*) genes in water samples was tested. The BPW water was inoculated with a selected STEC strain (STEC 210) at a concentration of 10^8 equivalent to an optical density (OD) value of 1. The sample was then serially diluted (10^0 - 10^8). Each dilution was plated out on duplicate L-EMB agar (Oxoid, South Africa). Colonies were counted after incubation at 24 h at 37°C. Each dilution was then screened for the presence of STEC (*stx* and *eae*) with the BAX assay and results were compared to colonies on the L-EMB plates.

Sample preparation

The DuPont™ BAX System Real-Time PCR assay (Hygiena) was used to screen for the STEC (*stx* and *eae*) genes. About 100 mL of water was filtered through sterile cellulose nitrate membrane filters with a pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa). Following filtration, the filters were transferred to 20 mL sterile Buffered Peptone Water (BPW) (Oxoid, South Africa) and incubated at 37°C for 24 h.

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BAX Assay

Following incubation, 20 µL of previously enriched sample was transferred to 200 µL of prepared BAX System lysis reagent (DuPont, South Africa) in cluster tubes. Lysis was performed according to the manufacturer's instructions by heating the tubes for 20 min at 37°C and 10 min at 95°C, and then cooling tubes at 4°C for at least 5 min, after which, 30 µL of the lysate was then transferred to PCR tubes containing reagent tablets. The PCR tubes were subsequently loaded into the BAX System Q7 instrument, and a fully automated process, which involved amplification and detection, was executed according to the manufacturer's instructions. Qualitative results were displayed as a grid of colour-cued icons at the top of the screen. Green (-) = negative for target organism and Red (+) = positive for target organism.

Ultraviolet Disinfection

UV Disinfection was conducted using a bench-scale collimated-beam device (Berson, The Netherlands), containing a LP UV lamp. The UV intensity at (254-nm wavelength) was measured using ILT1400 radiometer coupled with an XRL140T254 detector (International Light Technologies, USA). About 100 mL of water samples were exposed to UV light in 250 mL beakers for a specific time period to yield desired doses of 40 and 60 $mJ.cm^{-2}$ while continuously stirring the sample. The exposure time of germicidal UV light was calculated according to the equation of Hallmich & Gehr (2010) in order to deliver the desired UV dose.

$$I(\text{avg, } \lambda)\ (mW.\ cm^{-2}) = I0\lambda\ [\frac{1-e\text{dln}(\text{UVT}(\lambda))}{-\text{dln}(\text{UVT}(\lambda))}] \quad [1]$$

$$Desired\ dose\ (mJ.\ cm^{-2}) = Average\ intensity\ (mW.\ cm^{-2}) \times Exposure\ time\ (s) \quad [2]$$

In the equation above, I (avg, λ) is the average intensity of UV light over the sample depth, d; UVT(λ) refers to the UV transmission at wavelength, λ, determined using an optical path length of 1 cm; I0(λ) is the intensity of UV light measured at the surface of the sample.

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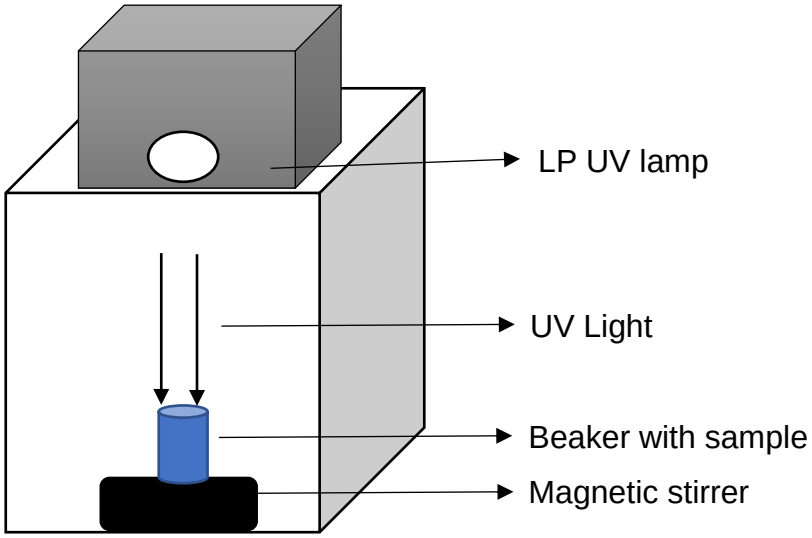

Figure 3 Representation of UV bench-scale irradiation used in this study

Recovery studies

Following UV disinfection, the potential for microbial recovery was investigated. In order to facilitate a potential optimum recovery, experiments were carried out in a controlled-environment (Figure 4). To simulate conditions for sunlight photoreactivation, fluorescent lamps (STR-GX3006A, South Africa) were used as the light source. The custom-made reactivation chamber contained two fluorescent lamps and a magnetic stirrer plate. A 250 mL beaker containing 100 mL of water sample was exposed to the light from the lamp and was continuously stirred with the aid of a magnetic stirrer for 3 h. The bacterial counts were then determined. Percentage recovery was calculated according to Guo *et al.* (2009), as follows:

$$\textit{Percentage recovery (\%)} = \frac{Np-N}{N0-N} \times 100\%$$

Here, N_P = cell number of recovered sample (CFU.mL^{-1}), N= immediate survival after UV disinfection (CFU.mL^{-1}), $N0$ =cell number before UV disinfection (CFU.mL^{-1}). The rates of recovery were assessed by determining microorganism survival from microbial numbers before disinfection and after recovery.

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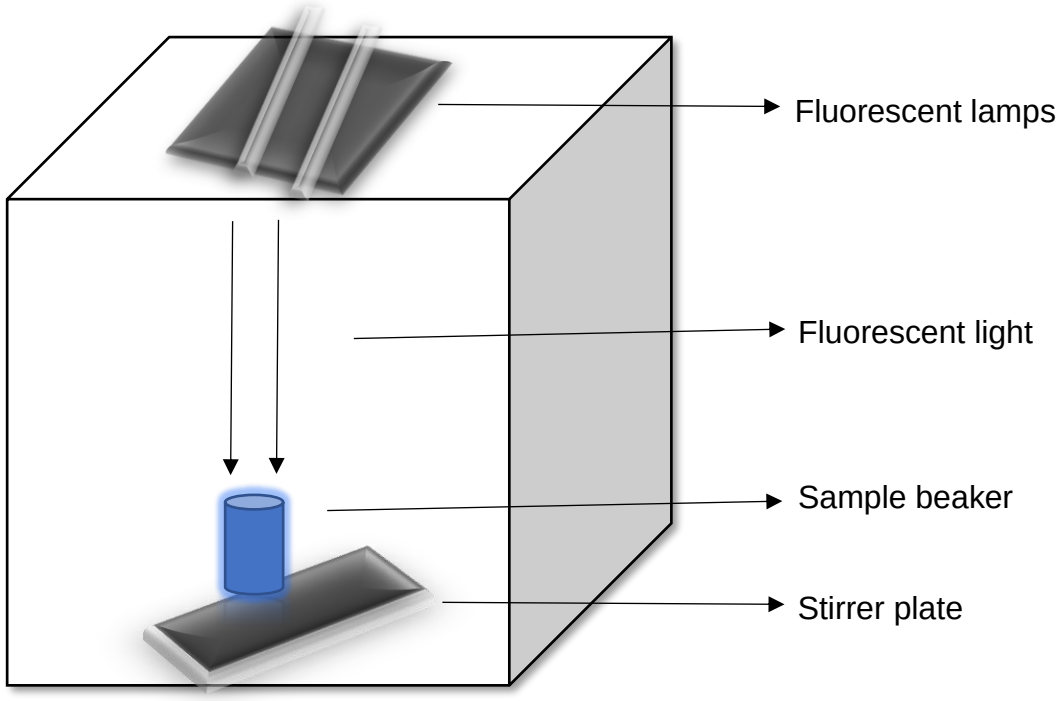

Figure 4 UV irradiated water samples exposed to fluorescent light for 3 h

Statistical analysis

Statistica 13.3 software (StatSoft, USA) was used to perform statistical analyses. The analysis was done using a two-way analysis of variance (ANOVA). Post hoc analyses were performed using the Fisher least significance difference (LSD) test and significant results were identified using a 95% significance level ($p<0.05$) as the guideline.

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Research study design

Several studies were performed to investigate the LP UV disinfection efficacy and the potential for microbial recovery on river water as can be seen in Figure 5.

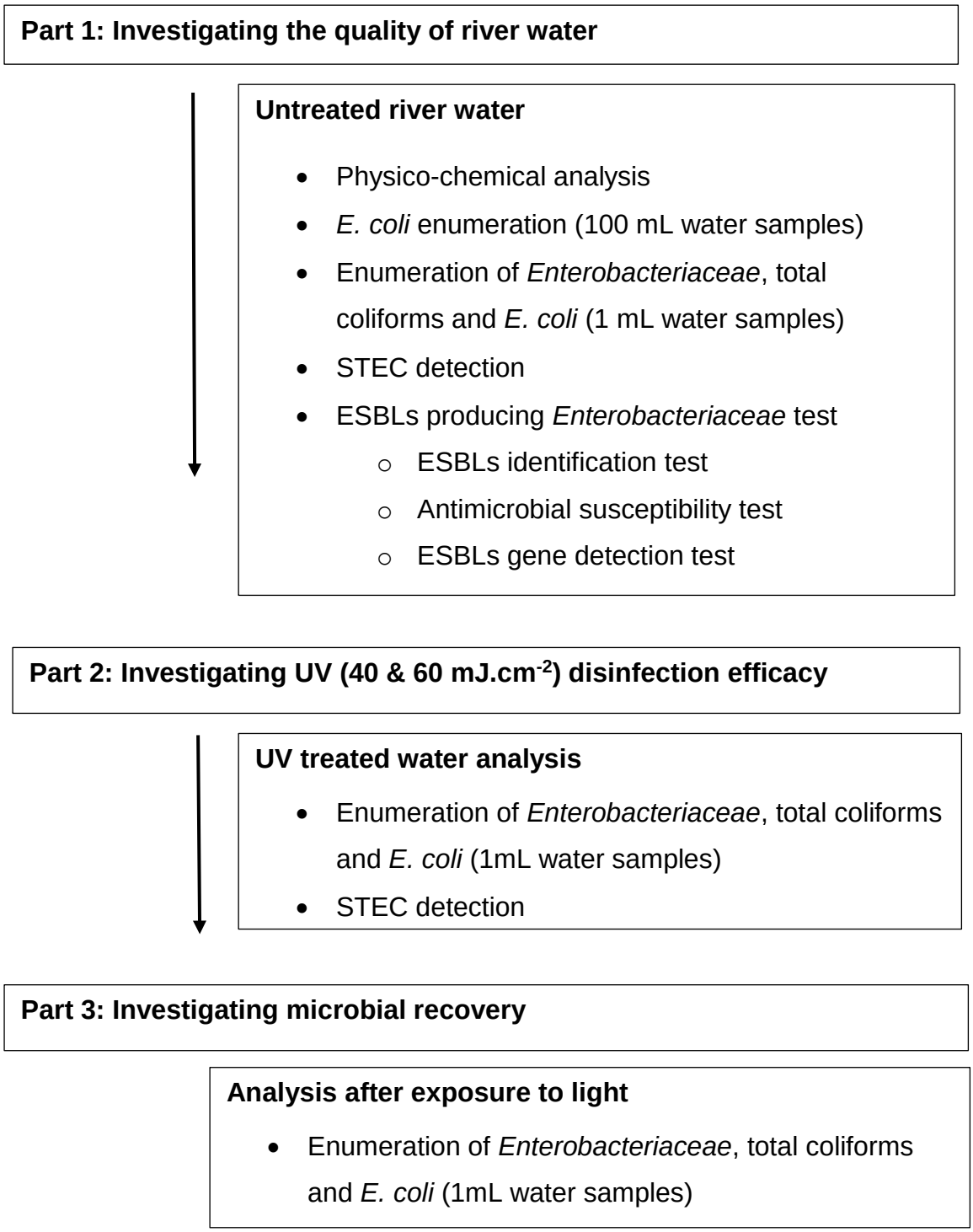

Figure 5 Flow diagram of experimental design followed in this study

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Part 1: Investigating the quality of river water

The water quality of three different rivers (Eerste, Krom and Plankenburg) was investigated before UV irradiation. This was done by assessing *E. coli* counts and the physico-chemical properties of the river water. *Escherichia. coli* was enumerated from 100 mL of water sample using the MF technique with Chromogenic coliform agar. Physico-chemical properties (in terms of alkalinity, COD, conductivity, turbidity, pH, TDS, TSS, UVT% and VSS) were measured. These results were compared to the irrigation standard guidelines set by DWAF (1996) to determine if the water is fit for irrigational purposes. The DWAF (1996), suggest a guideline limit of 1000 CFU. mL^{-1} of *E. coli* in irrigation water.

Due to the high *E. coli* counts reported in the Plankenburg River (Paulse *et al.*, 2009; Olivier, 2015), its association with pathogenic *E. coli*, and the health risks associated with exposure to *E. coli* pathotypes, the presence of STEC in the Plankenburg river water was investigated. The DuPont™ BAX System real-time PCR assay (Hygiena) was used according to the procedure described in the BAX System User Guide for the STEC *stx* genes. Further, because the risk associated with microbial contamination of water can be compounded by antibiotic resistance among pathogenic microorganisms, water samples in this study, were also screened for ESBL producing *Enterobacteriaceae.* Identification and confirmation of ESBL production was done as described under general materials and methods.

Part 2: Determining the LP UV disinfection efficacy of contaminated river water

In order to investigate the LP UV disinfection efficacy on river water, 25 mL of untreated water samples in 250 mL sterile glass beakers were exposed to UV light at two UV doses (40 and 60 $mJ.cm^{-2}$).

A collimated-beam device was utilised to execute the experiments. In order to interpret the effectiveness of the UV disinfection treatment, *Enterobacteriaceae*, total coliforms and *E. coli* were enumerated before and after UV disinfection using the pour plate technique and media (VRBG and Chromogenic Coliform agar) as described under general materials and methods. Where applicable, results were compared to the irrigation water guideline set by DWAF (1996). This was to determine if UV disinfection was able to reduce *E. coli* levels to acceptable irrigation levels.

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Enumeration of *Enterobacteriaceae* was also done as they are useful indicators of food safety and hygiene and of post-processing contamination of fresh produce.

In order to determine the effect of UV irradiation on STEC, as well as evaluate the UV sensitivity of different microorganisms, STEC was detected before and after UV disinfection treatments. Detection was carried out using BAX System real-time PCR assay (Hygiena) according to the procedure described under general materials and methods. Primarily, ESBLs can be produced by any member of the *Enterobacteriaceae* family. These species all have different UV resistances (Zimmer & Slawson, 2002; Yan *et al.*, 2017). For this reason, detection of ESBLs producers were not done on water samples after UV disinfection.

Part 3: Investigating the microbial recovery

The potential for irradiated populations to recover either as a result of photo repair or regrowth following LP UV irradiation was also investigated. River water samples irradiated at UV dose 40 and 60 mJ.cm^{-2} were exposed to fluorescent light for 3 h. The samples were continuously stirred using a magnetic stirrer and a stirrer bar (Figure 5). *Enterobacteriaceae*, total coliforms and *E. coli* were enumerated before reactivation (after UV disinfection) and directly after 3 h of exposure to the fluorescent light, using the pour plate technique and media (VRBG and Chromogenic Coliform agar) as described in general materials and methods. The increases in microbial numbers were expressed in terms of percentage recovery.

RESULTS AND DISCUSSION

Investigating the quality of river water

The general quality of river water was assessed by establishing the *E. coli* counts and physico-chemical properties of the Eerste, Krom and Plankenburg river water before UV disinfection. In addition, the occurrence of ESBL producers and STEC were also tested in the river water samples before UV disinfection.

The results presented in Table 3 show that the Eerste and Krom rivers conformed to irrigation guideline limits (DWAF, 1996). These rivers had *E. coli* counts of 1.81 and 2.01 log CFU.mL^{-1} respectively, which were within the stipulated regulatory guideline limit of 3 log CFU. mL^{-1} of *E. coli* in irrigation water (DWAF, 1996).

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The Plankenburg River was found to be highly contaminated with *E. coli* levels of up to 6.50 log CFU.mL^{-1}. Similar results were previously observed by other researchers. Paulse *et al*. (2009) reported *E. coli* counts of 6.54 log CFU.100 mL^{-1} in the Plankenburg River. Ndlovu *et al.* (2015) enumerated *E. coli* counts between 3 to 6.96 log CFU.100 mL^{-1} from the Plankenburg River. Olivier, (2015) found up to 6.41 log cfu.100 mL^{-1} in the Plankenburg River. These results demonstrate that the Plankenburg river water is not safe for fresh produce irrigation. The significantly higher counts of *E. coli* recorded for the Plankenburg River could possibly be attributed to untreated sewage discharge into the river from the informal settlement or the Plankenburg industrial area. The Kayamandi informal settlement (situated close to the Plankenburg River) is the major source of contamination (Paulse *et al*., 2009). The results in this study, suggest that *E. coli* reduction exceeding 3 logs would be required to yield water that is fit for irrigation purposes (*E. coli* < 3 log CFU.100 mL^{-1}). With regards to the *E. coli* guideline limit, researchers from the University of California from their irrigation water quality studies concluded that 3 log CFU in 100 mL^{-1} water sample was enough to minimise the chance of pathogens survival on fresh produce (Groves & Hulin, 2013).

The physico-chemical analysis revealed differences in quality parameters among the three different rivers and these are presented in Table 3. In general, physico-chemical parameters measured for the Eerste and Krom Rivers (including Alkalinity, COD, Conductivity, Turbidity, TDS, TSS, pH and VSS) were within the guideline limits recommended by DWAF, (1996) for irrigation purposes. Turbidity results indicated that both rivers (Eerste and Krom) did not contain a lot of solids (Table 3). Although the pH and TDS values of all river water samples were within range, the Plankenburg River water did not meet the guideline limits of COD, turbidity, TSS and VSS. Comparative COD measurements showed that the Plankenburg River had the highest COD value of all three rivers. Chemical oxygen demand is related to the presence of organic and inorganic pollutants in the water, which causes favourable conditions for the growth of microorganisms (Rajiv *et al.*, 2012). Water sampled from the Plankenburg River also represented the highest levels of TSS, VSS, conductivity and Turbidity (Table 1). High turbidity is expected to result in a low UVT% (Gayán *et al.*, 2014). With regards to UV treatment, low UVT% is generally expected to influence the efficacy of UV irradiation negatively (Wobma, 2004), by shielding microorganisms from the UV light (Edstrom., 2011). Physico-chemical variation of river waters are

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dependent on and influenced by the geographical location and climate change (Singh, 2013). The variations in water quality can significantly affect microbial inactivation by UV irradiation (Teksoy *et al.*, 2011).

Table 3 Quality properties of three different river water prior to UV disinfection and guidelines as recommended by DWAF (1996)

Quality parameters	Rivers			
	DWAF guidelines	**Eerste**	**Krom**	**Plankenburg**
Alkalinity	30 – 130	30	52	85
COD (mg. L^{-1})	10	10	7	35
Conductivity (mS.m^{-1})	≤ 40	7.6	20	41
Turbidity (NTU)	≤ 5	1.15	5	30
TSS (mg. L^{-1})	≤ 50	7	22	58
TDS (mg. L^{-1})	≤ 500	49	120	270
VSS (mg. L^{-1})	-	7.3	25	38
UVT%	> 75	86	71	27
pH	6.5 – 8.4	6.84	7.3	7.36
***E. coli* (log CFU. mL^{-1})**	≤ 3	1.81	2.01	6.50

Detection and isolation of Extended-Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae

Isolation of ESBL producing colonies

In this study, each of the three rivers was sampled three times. Each sample was screened for ESBL producing *Enterobacteriaceae* using the ChromID Brilliance ESBL agar (bioMérieux, South Africa). Based on the colour of colonies (*E. coli*: Pink/Burgundy; *Klebsiella/ Enterobacter/Serratia*: Blue/green; *Proteus*: light-dark brown), a total of 14 isolates from the Krom (*n=6)* and Plankenburg (*n=8)* rivers were considered presumptive ESBL producers. No ESBL producing *Enterobacteriaceae* were isolated from the Eerste River (Table 4).

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Table 4 Suspected prevalence of ESBL producing *Enterobacteriaceae* in three rivers in Stellenbosch

River	Growth on ESBL agar	Colony colour
Plankenburg	Positive	Burgundy, Green,
Plankenburg	Positive	Burgundy, Cream, Green
Plankenburg	Positive	Burgundy, Cream, Green
Krom	Positive	Burgundy
Krom	Positive	Burgundy, Cream, Green
Krom	Positive	Burgundy, Green
Eerste	Negative	N/A
Eerste	Negative	N/A
Eerste	Negative	N/A

Identification of ESBL producing Enterobacteriaceae

All 14 presumptive ESBL producing *Enterobacteriaceae* isolates from the Krom and Plankenburg Rivers were identified using MALDI-TOF mass spectrometry. The identities of the various species of microorganisms are shown in Table 5. Accordingly, *E. coli* (*n=7; 50 %*) and *Klebsiella pneumoniae (n=5; 35%)* were the most common ESBL producing *Enterobacteriaceae* recovered. The following organisms: *Pseudomonas otitidis* (*n=1; 7%*) and *Enterobacter asburiae* (*n=1; 7%*) were also identified amongst the isolates. These identification results corresponded with the colony colour observed during the ESBL screening test (Table 4). As indicated, most of the isolates were *E. coli* which were characterised by the pink/burgundy colour. This is a similar observation to those of other studies involving ESBL producing isolates in water, where the most commonly occurring species were *E. coli* and from the genus *Klebsiella* (Shehani & Lui, 2013; Guyomard-Rabenirina *et al.*, 2017). Given that most species of *Enterobacteriaceae* include commonly found enteric bacteria, these results suggest that the source of these bacteria might be faecal contamination from domestic sewerage and animal waste.

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Table 5 Isolate classification according to MALDI-TOF mass spectrometry

Microorganism	Number of isolates	Percentage (%)
E. coli	7	50
Enterobacter asburiae	1	7
Klebsiella pneumoniae	5	36
Pseudomonas otitidis	1	7

Antimicrobial susceptibility test (ESBL confirmation 1)

Extended-spectrum-beta-lactamases producing *Enterobacteriaceae* have increasingly emerged due to the widespread use of antimicrobials in human and veterinary medicine (Kittinger *et al.*, 2016; Vital *et al.*, 2018). Several studies have reported the increased prevalence of ESBL producing *Enterobacteriaceae* in the environment including surface water. (Rupp & Fey, 2003; Shehani & Lui, 2013; Kittinger *et al.*, 2016; Zarfel *et al.*, 2017; Vital *et al.*, 2018). Studies have also shown the presence of antibiotic-resistant bacteria in South African rivers (Romanis, 2013; Lamprecht *et al.*, 2014). This study was therefore carried out to investigate the prevalence of ESBL producing *Enterobacteriaceae* and antimicrobial resistance of *Enterobacteriaceae* in river water. The standard EUCAST ESBL confirmatory test revealed that 11 of the 14 (79%) ESBL presumptive-positive isolates showed a ≥ 5-mm increase in the growth-inhibitory zone diameter around CTX-CA, CAZ-CA and CPM-CA (Figure 5) and were thus considered positive for Group 1 and Group 2 ESBL production (Table 6). During the antibiotic susceptibility testing, antibiotics discs were placed on an inoculated MHA plate and incubated. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the disc where the bacteria have not grown enough to be visible (Brown & Kothari, 1975). This is called a zone of inhibition. If the zone of inhibition of the test strain is within the area marked with an ‘R’, the organism is resistant. If the zone of inhibition is equal to or larger than the marked area, the organism is susceptible (Howe & Andrews, 2012).

The resistance of the 14 isolates against three other antibiotics is also summarised in Table 6. Amongst the river water isolates tested in this study, the highest resistance (100%) was observed in Ampicillin, followed by resistance to Tetracycline (79%). Resistance to Chloramphenicol was 21%. Vital *et al.* (2018), observed similar results regarding the antimicrobial susceptibility of *E. coli* isolates

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from water. In their study, the highest resistance was observed against Tetracycline and Ampicillin. Lamprecht *et al*. (2014) in their study, also found Ampicillin (5/5) and Tetracycline (3/5) to be some of the antibiotics that their *E. coli* water isolates were most resistant to. The presence of Tetracycline-resistant *Enterobacteriaceae* in irrigation waters is a major concern, especially in some countries, where Tetracycline is widely used as a first-line drug in the treatment of gastrointestinal infections (Vital *et al.*, 2018). The indiscriminate use of antibiotics in clinics and animal production could have contributed to the extent to which resistance has spread in the environment (Lamprecht *et al.*, 2014).

Important to note is that multidrug resistance (MDR) bacteria were also observed. Multidrug resistance is defined as the resistance of bacteria to at least three different classes of antibiotics (Magiorakos *et al.*, 2012). In this study, 2 of 14 (14%) isolates were MDR (Table 6). Isolates in this study were only tested against three classes of antimicrobials. They may, therefore, be resistant to other classes of antimicrobials such as aminoglycosides, fluoroquinolones and cephalosporins which were not tested. Similar results were observed by Lamprecht *et al.*, (2014) where some of the isolates from the Plankenburg river were found to be MDR. Further, Roe *et al*., (2003) showed that the Rio Grande River, a major source of irrigation water for both the USA and Mexico, harbours MDR *E. coli* with a prevalence rate of 32%. From this study, it was observed that the MDR prevalence was only in the Plankenburg River. A plausible explanation could be the fact that, the Plankenburg River is highly contaminated compared to the other two rivers. Antibiotics and antibiotic-resistant bacteria (ARB) in the environment stem from many different sources like hospital effluents, informal communities, industry and farming which all end up in surface waters (Kittinger *et al.*, 2016). Multidrug resistance in bacteria occurs by the accumulation of genes on resistance plasmids (Roe *et al.*, 2003). The emergence of antibiotic resistance outside the clinical setting and especially in aquatic environments, such as surface waters is well documented (Rupp & Fey, 2003; Kittinger *et al.*, 2016; Vital *et al.*, 2018).

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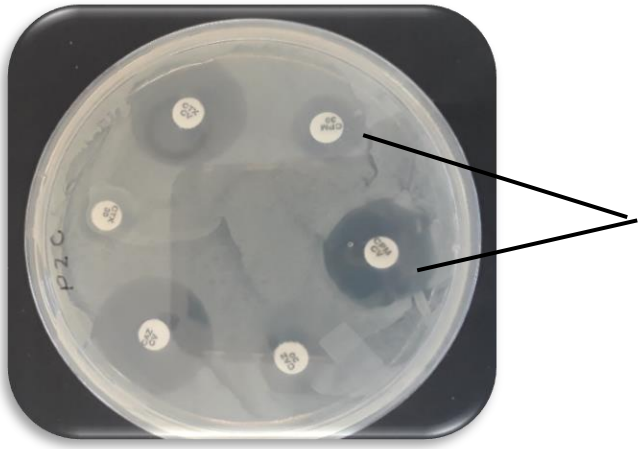

Figure 6 Example of a Mueller-Hinton Agar plate inoculated with an ESBL producing positive strain

Table 6 Shows ESBL production and antibiotic susceptibility profiles of fourteen ESBL producing isolates from the Krom and Plankenburg Rivers

Increase in growth-inhibitory zone diameter				ESBL producer (Yes/No)	Antibiotic susceptibility			MDR (Yes/No)
Organisms code	**CTX**	**CAZ**	**CPM**		**AMP**	**C**	**TE**	
ATCC 25922					S	S	S	No
ATCC 35218					R	R	R	Yes
K1B	17	10	13	Yes	R	S	R	No
K2B	15	14	12	Yes	R	S	R	No
K2C	1	1	1	No	R	S	R	No
K2G	11	9	8	Yes	R	S	R	No
K3B	14	11	6	Yes	R	S	R	No
K3G	5	2	1	No	R	S	S	No
P1B	0	1	1	No	R	S	R	No
P1G	11	11	10	Yes	R	R	R	Yes
P2C	13	12	8	Yes	R	S	R	No
P2B	15	13	10	Yes	R	S	R	No
P2G	13	8	8	Yes	R	R	S	No
P3B	15	14	8	Yes	R	S	S	No
P3C	19	11	8	Yes	R	S	R	Yes
P3G	10	7	8	Yes	R	R	R	Yes

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R=Resistant, S=Sensitive, CTX=Cefotaxime, CAZ= Ceftazidime, CPM= Cefepime, AMP= Ampicillin, C= Chloramphenicol, TE= Tetracycline

Detection of ESBL Genes (ESBL confirmation 2)

In this study, 14 bacterial isolates including seven *E. coli and five Klebsiella pneumonia* were analysed (Table 5). Amongst these 14 isolates, 11 (79%) were confirmed as ESBL producers with the disc-based EUCAST ESBL confirmatory test while three (21%) were confirmed non-ESBL producers (Table 6). All isolates were subjected to PCR analysis. Of the three beta-lactamases (*bla*) genes studied, CTX-M was detected in 13 (92 %) isolates, followed by TEM in 12 (86 %) isolates and SHV in four (28 %) isolates (Figure 6). Most of the *E. coli* (50 %) isolates in this study, carried the CTX-M genes while most of *Klebsiella* isolates were found to be co-producers of the ESBL genes; either two or all the three genes occurred together. The *Enterobacter* isolate showed the presence of the TEM genes only (Table 7). Similar to these findings, a high prevalence of CTX-M has been reported in water isolates before. Shehani & Lui, (2013) found CTX-M (84.2%) and TEM (47.4%) to be the predominant *bla* genes in surface water isolates. Recently, CTX-M gene was present in 29 of 33 ESBL producing strains isolated from wastewater (Guyomard-Rabenirina *et al.*, 2017). The CTX-M and the SHV genes together were found in 4 (28 %) isolates (Figure 6). The TEM and SHV genes together were also found in 4 (28%) isolates while the CTX-M and the TEM genes together were found in 12 (85%) isolates. In this study, 4 (28%) isolates, contained all the three *bla* genes (CTX-M, SHV and TEM) (Table 7). Co-existence of *bla* genes in isolates from river water has been reported (Maravić *et al.*, 2015).

The prevalence of CTX-M -type β-lactamase in most isolates is quite worrisome. Unlike TEM-1 and SHV-1, which are broad-spectrum β-lactamases that confer resistance to penicillins and first-generation cephalosporins, but not to third- and fourth-generation cephalosporins (Stadler *et al.*, 2018), plasmids carrying *bla*CTX-M genes frequently carry other antibiotic resistance determinants such as plasmid-mediated quinolone resistance (Guyomard-Rabenirina *et al.*, 2017). And may therefore confer resistance to many classes of antibiotic. The epidemic dissemination of CTX-M-encoding genes is largely due to their locations on mobile genetic elements, such as plasmids, transposons and integrons (Rupp & Fey, 2003), which allow these genes to readily spread among bacterial communities. Thus, the large variety of

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species recovered from river waters that carry this gene points to the possible horizontal transfer of blaCTX-M via conjugative plasmids in this water environment (Shehani & Lui, 2013).

In terms of risk to human health, studied have highlighted that potential ESBL species such as *K. pneumonia* and *E. coli* have a high tendency to possess and transfer *bla* genes (Shehani & Lui, 2013; Stadler *et al.*, 2018). Transfer of genes may occur by conjugation because the genes are often found on mobile elements like transposons and integrons (Stadler *et al.*, 2018). Some of these species may be pathogenic strains that have the potential to cause life-threatening diseases and widespread outbreaks. For instance, *bla*CTX-M and *bla*TEM genes in opportunistically pathogenic *Klebsiella* spp. have been associated with nosocomial infections and outbreaks of diarrhoea (Shehani & Lui, 2013).

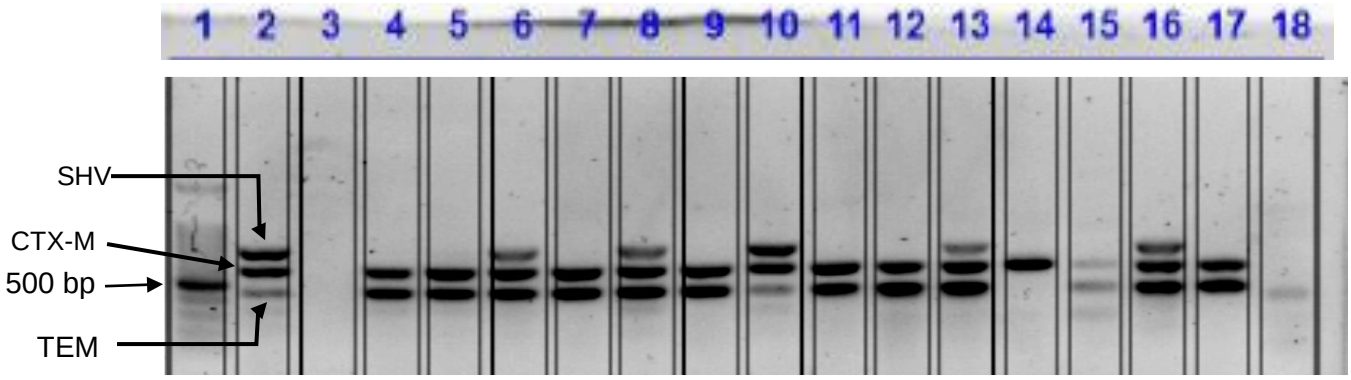

Figure 7 Gel pictures of amplified PCR products (SHV gene: 747 bp, TEM gene: 445 bp, CTX-M gene: 593 bp) and ladder; Samples, 1 = ladder, 2 &10 = Positive control, 3 = Negative control, 4 = K1B, 5= K2B, 6= K2C, 7=K2G, 8=K3B, 9=K3G, 11= P1B, 12=P1G, 13=P2C, 14=P2B, 15=P2G, 16=P3B, 17=P3C,18=P3C.

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Table 7 Distribution of TEM, SHV and CTX-M ESBL types among 14 isolates studied.

Presence of ESBL	Total	*E. coli* (*n=7*)	*Klebsiella pneumoniae* (*n=5*)	*Pseudomonas Otitidis*	*Enterobacter*
TEM	12	6	5	0	1
SHV	4	2	2	0	0
CTX-M	13	7	4	1	0
CTX-M +SHV	4	2	2	0	0
TEM +SHV	4	2	2	0	0
CTX-M + TEM	12	7	5	0	0
CTX-M + TEM + SHV	4	4	0	0	0

Table 8 Identified organisms with ESBL genes

Sample code	Organisms	ESBL gene
K1B	*Escherichia coli*	TEM, CTX-M
K2B	*Escherichia coli*	TEM, CTX-M
K2C	*Escherichia coli*	TEM, CTX-M and SHV
K2G	*Klebsiella pneumoniae*	TEM, CTX-M
K3B	*Escherichia coli*	TEM, CTX-M and SHV
K3G	*Enterobacter asburiae*	TEM
P1B	*Klebsiella pneumoniae*	TEM, CTX-M
P1G	*Pseudomonas otitidis*	CTX-M
P2C	*Escherichia coli*	TEM, CTX-M and SHV
P2B	*Escherichia coli*	TEM, CTX-M
P2G	*Klebsiella pneumoniae*	TEM, CTX-M
P3B	*Escherichia coli*	TEM, CTX-M and SHV
P3C	*Klebsiella pneumoniae*	TEM, CTX-M
P3G	*Klebsiella pneumoniae*	TEM, CTX-M

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BAX system validation results

In order to evaluate the sensitivity of the DuPont™ BAX System Real-Time PCR assay (Hygiena) to detect STEC (*stx* and *eae*) genes, BPW was inoculated with a selected STEC (STEC 210) strain. The detection limit for STEC 210 enrichment was 27 CFU.mL^{-1}. BAX system was unable to detect the STEC 210 in a suspension of < 27 CFU.mL^{-1}.

Determining the LP UV disinfection efficacy of river water

UV irradiation is known to effectively inactivate a wide range of microorganisms (Hijnen *et al.*, 2006). To evaluate its effectiveness in this study, river water from three different rivers (Eerste, Krom and Plankenburg) was exposed to two different UV doses (40 and 60 mJ.cm^{-2}). The choice of the UV doses was based on literature review and findings from Chapter 3 of this thesis. It has been reported that a dose of 40 mJ.cm^{-2} is enough to cause a 4-log reduction of *E. coli* while preventing reactivation (Zimmer & Slawson, 2002). In Chapter 3 of this thesis, this dose achieved more than a 4-log reduction of the different *E. coli* strains (n=5) tested. Before and after each disinfection treatment, all samples were tested for the presence of STEC while three populations (*Enterobacteriaceae*, total coliforms and *E. coli*) were enumerated.

Results presented in Table 9, indicate a substantial decrease in population size following exposure to UV irradiation at both doses (40 and 60 mJ.cm^{-2}). For the Plankenburg River, microbial counts at dose 40 mJ.cm^{-2} decreased from 5.29 to 1.78 log CFU.mL^{-1}, from 5.36 to 1.80 log CFU.mL^{-1} and from 5.22 to 1.54 CFU.mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli*, respectively. A further reduction in population size was observed at a higher UV dose (60 mJ.cm^{-2}) (Table 9). In this case, microbial counts decreased from 5.29 to 1.22 log CFU.mL^{-1}, from 5.36 to 1.36 log CFU.mL^{-1} and from 5.22 to 1.08 CFU.mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli*, respectively. For the Eerste and Krom rivers, bacterial counts were below detectable levels following exposure to UV at both doses (Table 9). Log reductions were therefore only calculated using results from the Plankenburg River and are presented in Figure 8.

Average log reductions at UV dose 40 mJ.cm^{-2} were 3.37, 3.33 and 3.51 log for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively and 3.79, 3.77 and 4.03 log for *Enterobacteriaceae*, total coliforms and *E. coli* respectively at dose

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60 mJ.cm^{-2}. As stated in the previous chapter, a suggested 3-log reduction of *E. coli* can provide water that is suitable for irrigational purposes, should the initial *E. coli* concentration be around 10^4 - 10^5 CFU.100 mL^{-1} (Britz *et al.*, 2013; Olivier, 2015). As shown in Table 9, a 3-log reduction was able to reduce the *E. coli* counts to acceptable levels (<3 log CFU.100 mL^{-1}).

Table 9 Bacteria count (log CFU. mL^{-1} ± standard deviation) of river water samples before and after UV disinfection.

		UV doses (mJ.cm^{-2})	
River	**Before UV**	**40**	**60**
Enterobacteriaceae			
Plankenburg	5.29±0.42	1.78±0.35	1.22±0.44
Krom	2.06±0.06	0	0
Eerste	1.00±0.21	0	0
Total coliforms			
Plankenburg	5.36±0.46	1.80±0.31	1.36±0.54
Krom	2.17±0.04	0	0
Eerste	1.18±0.15	0	0
E. coli			
Plankenburg	5.22±0.45	1.54+0.43	1.08+0.35
Krom	1.08±0.06	0	0
Eerste	0.30±0.13	0	0

Even though increased log reductions of all microorganisms were observed at UV dose 60 mJ.cm^{-2}, the difference from the log reduction observed at UV dose 40 mJ.cm^{-2} was not statistically significant (P>0.05) (Figure 8). For *E. coli*, the difference in log reduction between dose 40 and 60 mJ.cm^{-2} was 0.35 log. It was further observed that the differences in log reduction among microbial populations at both UV doses (40 & 60 mJ.cm^{-2}), were also not significant (P>0.05). At a UV dose of 40 mJ.cm^{-2}, the difference in log reduction between *Enterobacteriaceae* and total coliform was 0.03 log, while the difference was 0.01 log at dose 60 mJ.cm^{-2}. The highest increase in log reduction (from dose 40 to 60 mJ.cm^{-2}) was however achieved for *E. coli*, with *Enterobacteriaceae* and total coliforms having very similar log increments (Figure 8). These observed results could partly be because the *E. coli* count before UV irradiation was significantly lower than that of *Enterobacteriaceae* and total coliforms, making it

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easier to inactivate. A small bacterial population in a sample is likely to result in the full exposure of bacteria to UV light (Gayán *et al.*, 2013a). Another reason could be because, *Enterobacteriaceae* and total coliform group are a heterogeneous population that involves various strains and species that may be more resistant to UV than *E. coli* (Guo *et al*., 2009). Like these findings, Guo *et al*, (2009) in their study also found total coliforms to be more UV resistant when compared to *E. coli*.

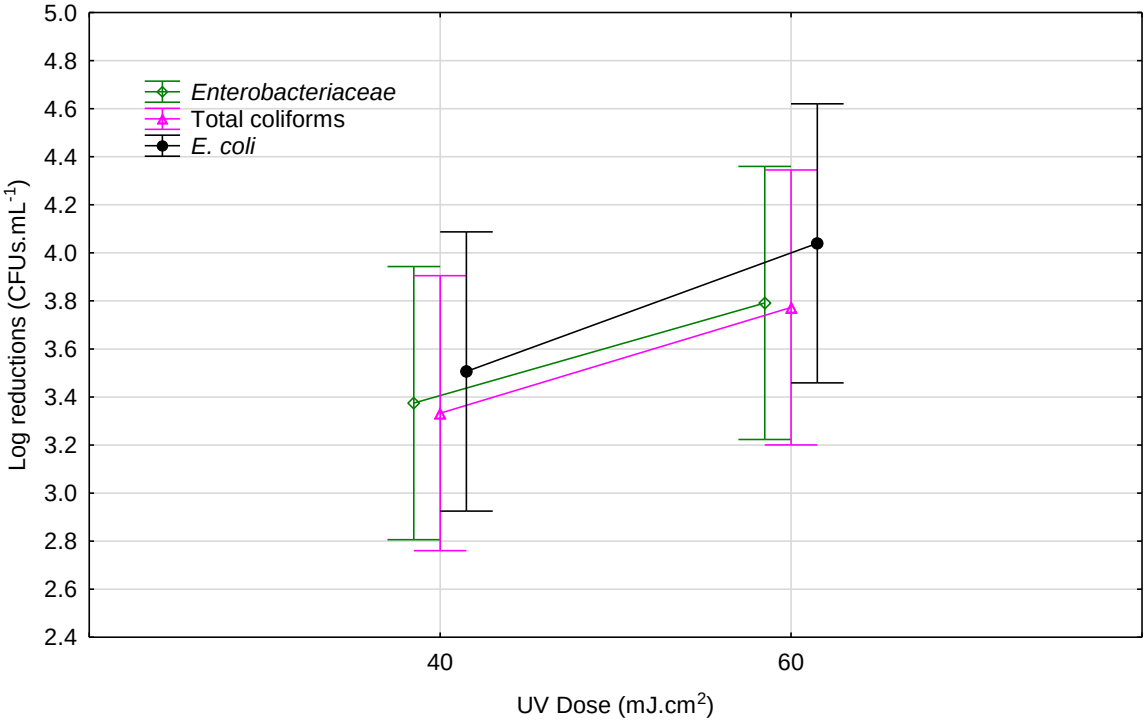

Figure 8 Log reductions of microorganisms achieved at two doses (40 and 60 mj.cm^{-2}). Error bars were calculated based on standard deviation at a confidence interval of 0.95

Considering the influence of physico-chemical parameters, it was not clear in this study if particles in the water influenced UV disinfection efficiency by blocking and/or scattering the UV light. The total reductions of microbial populations achieved for the Eerste and Krom Rivers could not really be attributed to low levels of suspended and dissolved solids in these rivers because the initial bacterial counts in these rivers were low, making them very easy to inactivate. Likewise, the 3-log reduction (not total reduction) achieved for the Plankenburg River could also not entirely be attributed to the high levels of suspended and dissolved solids in this river but could be due to the initial high microbial counts. The negative impact of suspended and dissolved compounds on UV light was thus not clear in this study. Like these findings, Olivier,

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(2015) could also not conclude if water quality parameters had an influence on UV disinfection efficacy citing that the influence of scattering by particles on UV is more complex than simply referring to differences in concentrations of the influential particles.

What was apparent in this study, was the effects that water quality parameters had on UVT%. An inverse relationship between some quality parameters (TSS, TDS and turbidity) and UVT% was observed. For the Plankenburg River, high turbidity due to high TDS and TSS resulted in a low UVT%. Low UVT% translated to longer UV exposure time in order to achieve the required doses.

Effects of UV irradiation on STEC

The presence of STEC in each water sample was tested before and after UV disinfection. Table 10 shows the occurrence of STEC in these samples. Four of nine (44 %) samples tested positive for STEC before UV irradiation. Representing 3 of 3 (100 %) from the Plankenburg River, 1 of 3 (33%) from the Krom and none from the Eerste. These results strongly suggest the prevalence of STEC in the Plankenburg River. The prevalence of STEC in surface waters has been reported by other researchers (Ram *et al.*, 2011; Beutin & Martin, 2012; Ennis *et al.*, 2012). Ndlovu *et al.* (2015) reported the presence of STEC genes in the Berg River in South Africa. In terms of the Plankenburg River specifically, Britz *et al.* (2013) reported the frequent presence of other pathogens (*Staphylococcus spp*. and *Listeria spp*) in the Plankenburg River water samples. Lamprecht *et al.* (2014) also reported the presence of other pathogenic (Enteroaggregative and Enteropathogenic) *E. coli* strains in the Plankenburg River. No reports of the presence of STEC in the Plankenburg has been made before.

A conceivable cause for the high incidence of STEC in the Plankenburg River in this study, may be due to the constant contamination of the water. The Plankenburg River is a source of water for various activities in the Kayamandi informal area (Paulse *et al.*, 2009). These may, in turn, contribute to the point or non-point source contamination of this river (Paulse *et al.*, 2012). The detection of STEC in the Plankenburg may also be an indication of recurrent faecal contamination. This finding, in itself, is not surprising because it is well established that the Plankenburg River carries high levels of *E. coli* (Paulse *et al.*, 2012; Britz *et al.*, 2013; Sigge *et al.*, 2016).

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High levels of indicators are known to correlate with the possible presence of pathogens (Tallon *et al.*, 2005; Truchado *et al.*, 2017).

Regarding the once off STEC detection in the Krom River (Table 10), it is unclear whether the Krom River harbours STEC or not. Inconsistent STEC prevalence suggests that hydrological factors such as rainfall or sporadic contamination likely played an important role in the occurrence of STEC in the Krom river water. This result suggests the need to further monitor STEC in this river. The low levels of *E.coli* in the Krom River also confirms that the level of indicator organisms may not necessarily correlate with the presence or absence of pathogens (Tallon *et al.*, 2005). No STEC was detected in the Eerste River.

STEC detection results after UV disinfection at doses 40 and 60 mJ.cm^{-2} are also presented in Table 10. Observations were that two samples tested positive for STEC at a UV dose of 40 mJ.cm^{-2}. It was further observed that all samples tested STEC negative at UV dose 60 mJ.cm^{-2} (Table 10). The results suggest that a UV dose of 40 mJ.cm^{-2} may not be enough to inactivate these STEC. Environmental STEC has been reported to be resistant to low UV doses (Mofidi *et al.*, 2002; Sigge *et al.*, 2016). Yan *et al.* (2017) found that to achieve a log reduction of 4.54–5.31 for pathogenic *E. coli*, the UV dose should at least be 51 mJ.cm^{-2}.

Table 10 STEC results of river water samples before and after UV disinfection

River	Before UV	40 mJ.cm^{-2}	60 mJ.cm^{-2}	STEC genes
Plankenburg	Positive	Not tested	Not tested	*stx,eae*
Plankenburg	Positive	Positive	Negative	*stx,eae*
Plankenburg	Positive	Positive	Negative	*stx,eae*
Krom	Positive	Not tested	Not tested	*stx,eae*
Krom	Negative	Negative	Negative	N/A
Krom	Negative	Negative	Negative	N/A
Eerste	Negative	Not tested	Not tested	N/A
Eerste	Negative	Negative	Negative	N/A
Eerste	Negative	Negative	Negative	N/A

Investigating the microbial recovery after UV irradiation

UV irradiation is a well-established water treatment method. It is, however, well-known that many microorganisms including total and faecal coliform have the ability to repair

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UV-induced damage (Nebot Sanz *et al.*, 2007; Salcedo *et al.*, 2007; Guo *et al.*, 2009). To investigate the repair or regrowth potential of *Enterobacteriaceae*, total coliforms and *E. coli*, UV disinfected water samples were exposed to 3 h of fluorescence light. Results were expressed as percentage recovery. The time of exposure to light was based on findings from previous studies. Olivier (2015) from his study observed that maximum recovery of total coliforms following UV disinfection was achieved in the 1st hour of a 5 h exposure to light. Recovery decreased after 3 h of exposure. Quek & Hu (2008) also reported that repair of *E. coli* strains generally occurred rapidly within the first hour of fluorescent light exposure, followed by a levelling off, of the recovery. Results from the Eerste and Krom Rivers will not be presented, as no counts were observed following recovery experiments.

Table 11 shows microorganism log count of the Plankenburg River before and after reactivation. From the results, UV inactivated microorganisms had the ability to multiply and re-contaminate the disinfected water during exposure to fluorescence light. This was observed at both UV doses (40 and 60 mJ.cm^{-2}). After a UV dose of 40 mJ.cm^{-2} followed by 3 h exposure to light, microbial population size increased from 1.78 to 2.89 log CFU. mL^{-1} from 1.80 to 2.63 CFU. mL^{-1} and from 1.54 to 2.32 CFU. mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. At a dose of 60 mJ.cm^{-2}, the population increase was from 1.22 to 1.85 log CFU. mL^{-1}, from 1.35 to 1.81 log CFU. mL^{-1} and from 1.54 to 1.73 log CFU.mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. Even though the UV disinfected water samples were not exposed to dark repair conditions in this study, it was speculated that recovery might have been due to photo repair or enhanced re-growth due to extra nutrients. Olivier (2015), in his study, found that most microbial growth resulted from light-induced DNA repair. Ultraviolet irradiation does not show any residual activity in water following the disinfection, that can inhibit growth. Extra nutrients that were released during the cell lysis that occurred after irradiation may aid any remaining bacteria to grow (Freese & Nozaic, 1999)

The percentage recovery of microorganisms was compared at the two respective doses as shown in Figure 9. Percentage recovery was less than 1% at both UV doses (40 & 60 mJ.cm^{-2}). When a germicidal UV dose of 40 mJ.cm^{-2} was applied, recovery percentage was 0.61, 0.35 and 0.20 % for *Enterobacteriaceae*, total coliforms and *E. coli* respectively. While the recovery percentage at dose 60 mJ.cm^{-2} was, 0.11, 0.10 and 0.01% for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. Even

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though percentage recovery did not exceed 1%, this low percentage would produce a recovery of 10^4 colonies if the initial number of colonies before UV are around 10^6 CFU.mL^{-1}, which would still make UV treated water unfit for agricultural irrigation.

The results (Figure 9) showed that the level of recovery is certainly variable depending on UV doses. Clearly, the level of recovery was lower following irradiation at the higher UV dose (60 mJ.cm^{-2}) (Figure 9). For *Enterobacteriaceae*, the percentage recovery was 0.5% less at dose 60 mJ.cm^{-2}. Similar trends were seen by previous researchers. Olivier, (2015) reported that higher UV doses resulted in less recovery. In his study, a significantly lower percentage recovery (35.37%) was observed when a UV dose of 24 mJ.cm^{-2} was applied as compared to a percentage recovery of 49.18% when a dose of 13 mJ.cm^{-2} was applied. When Guo *et al.* (2009) investigated the repair potential of *E. coli* and total coliforms in wastewater, they found that the recovery of total coliforms was 50% when a UV dose of 15 mJ.cm^{-2} was applied. When a higher UV dose of 40 mJ.cm^{-2} was applied the recovery was less than 1%. It was argued that exposure to a higher UV dose could induce more pyrimidine dimers, which are numerous enough to inhibit repair that could occur within a specific time frame (Guo *et al.*, 2009). The results in Figure 9 also demonstrate that *E. coli* showed recovery to a lesser extent in comparison to *Enterobacteriaceae* and total coliforms. The differences in recovery achieved among these populations at both UV doses were, however, not statistically significant (P>0.05). This could be attributed to the fact that *E. coli* counts were lower before reactivation and that *E. coli* had the highest log reduction (Table 11). Higher log reductions may lower the extent of repair that could occur (Guo *et al.*, 2009). The difference in percentage recovery between *Enterobacteriaceae* and total coliforms was not statistically significant (P>0.05).

Table 11 The microorganism count (log CFU. mL^{-1} ± standard deviation) of the Plankenburg river before (after UV) and after reactivation (3 h).

		UV disinfection		**Photoreactivation**	
Populations	Before UV	40	60	40	60
Enterobacteriaceae	5.29±0.42	1.78±0.35	1.22±0.44	2.89±0.66	1.85±0.14
Total coliform	5.36±0.46	1.80±0.31	1.35±0.54	2.63±0.37	1.81±0.11
E. coli	5.22±0.45	1.54±0.43	1.08±0.35	2.32±0.11	1.73±0.02

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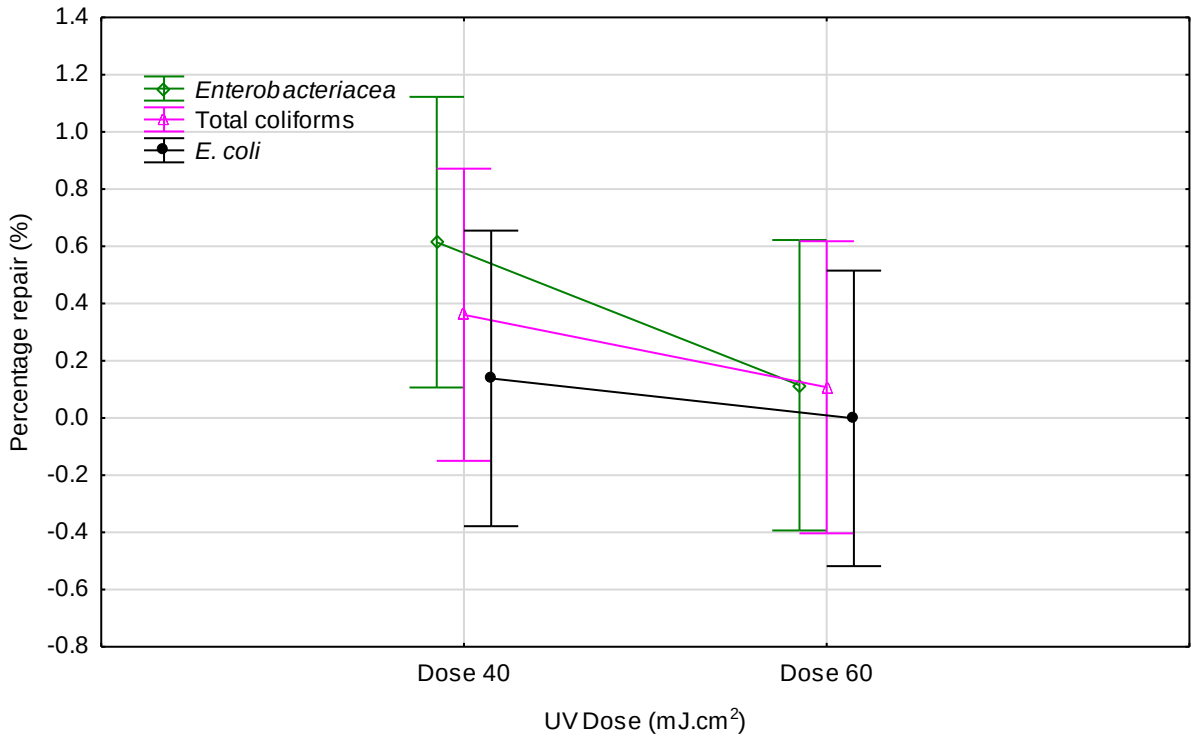

Figure 9 Percentage recovery of microorganisms achieved at two doses (40 and 60 mJ.cm^{-2}). Error bars were calculated based on standard deviation at a confidence interval of 0.95.

CONCLUSION

Comparative water quality analysis on river water showed that the Eerste River was the least polluted river investigated in this study. Water from this river complied with guidelines for irrigation water set for physico-chemical and microbiological standards (DWAF, 1996), and it was concluded that the water from the Eerste River could be used for irrigation of fresh produce without prior treatment.

Water from the Krom River was, in terms of physico-chemical characteristics and *E. coli* counts, of acceptable quality for irrigation. However, the presence of ESBL producing *Enterobacteriaceae* and the sporadic occurrence of STEC raised a question of whether the Krom River is fit for irrigation of fresh produce or not. Further monitoring of this river in future is, therefore, recommended. The presence of STEC and ESBL producers despite the low *E. coli* counts in this river, also raised another important question of whether the standard irrigation guideline of < 3 log CFU.100 mL^{-1} of *E. coli*

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is safe enough. Due to health risks associated with ESBL producers that can transfer mobile resistance elements to pathogens if present, this study recommends irrigation standard guidelines to include pathogens such as STEC, *Salmonella*, *Listeria monocytogenes* and *Campylobacter jejuni*, which present a key concern for surface contamination of fresh produce in addition to monitoring *E. coli*. It was concluded from this study that, the presence of low level of indicators organisms does not necessarily rule out the presence of pathogenic organisms. Indicators alone can, therefore, not always be considered an indication of the presence or absence of other possible pathogens.

The Plankenburg River was highly polluted and displayed *E. coli* levels up to 6.50 log CFU.100 mL^{-1}. The findings also showed the presence of STEC and ESBL producers in the Plankenburg River. This suggests that the use of the river water for irrigation of fresh produce could pose serious health risks. It is, therefore, in the public interest to treat this water before use. UV disinfection resulted in at least a 3-log reduction for all microorganisms at both UV doses (40 and 60 $mJ.cm^{-2}$). For less contaminated water from the Eerste and Krom Rivers, UV irradiation resulted in undetectable levels of all microorganisms. For highly contaminated water from the Plankenburg River, a 3-log reduction was able to reduce the *E. coli* level in contaminated water to current acceptable irrigation standards. These results confirm that UV is indeed an effective water disinfection method in terms of indicator organisms. When considering the inactivation of STEC, it was observed that a UV dose (40 $mJ.cm^{-2}$) was inadequate to inactivate the STEC. It was thus concluded that the UV dose should at least be 60 $mJ.cm^{-2}$ to achieve significant inactivation of both indicator organisms and STEC.

When considering the impact of water quality on UV disinfection, this study, could not clearly establish whether suspended particles shielded microorganisms from the UV light. What was clear though was that, the presence of suspended and dissolved particles in water had an important effect of reducing UVT% which required significantly longer UV exposure times to achieve the desired UV dosages. These findings strongly suggest pre-treatment of contaminated water before UV disinfection in order to reduce contact times and improve UV disinfection efficiency.

This study also confirmed repair of microorganism following LP UV disinfection. repair was observed in UV treated river water at both UV doses. Although recovery was less than 1% at both doses, in highly contaminated water, this 1% recovery can

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result in microbial population size exceeding the stipulated guideline limit for water used for irrigational purposes. It was also observed that the higher UV dose of 60 mJ.cm^{-2} resulted in lower levels of repair compared to 40 mJ.cm^{-2}. Higher germicidal UV doses would, therefore, be expected to progressively inhibit repair. Thus, when recovery of microorganisms after exposure to UV in highly contaminated river water is considered, UV doses higher than 60 mJ.cm^{-2} should potentially be considered.

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CHAPTER 5

EVALUATING THE DISINFECTION EFFICACY OF PINE BIOCHAR FILTRATION AND ULTRAVIOLET (UV) IRRADIATION AND COMBINATION TREATMENT ON REDUCING MICROBIAL LOADS IN RIVER WATER

ABSTRACT

The disinfection efficacy of a combination treatment involving pine biochar filtration and UV irradiation was tested on the Plankenburg River water. Pine biochar filtration was used as a pre-treatment to improve the physico-chemical parameters of river water. In order to determine the extent of microbial reduction, each treatment was used as a stand-alone treatment and in combination. Pine biochar filtration was able to reduce the chemical oxygen demand (COD), turbidity, total suspended solids (TSS) and volatile suspended solids (VSS) of the river water and resulted in an improved UVT%. With regards to microbial reduction, UV irradiation alone was able to reduce microorganisms to undetectable levels when the initial microbial counts were low (Trials 1 and 4). When the initial microbial counts were high (Trials 2 and 3), log reductions ranged between 3.06 – 3.18, 2.02 – 2.36 and 2.22 – 2.33 log CFU.mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. It also effectively inactivated STEC to below detectable levels when a UV dose of 40 mJ.cm^{-2} was applied. Pine biochar filtration was unable to remove STEC and reduce microorganisms to meet the irrigation standard guidelines limit of 1 000 CFU.ml^{-1} *E. coli*. Log reductions ranged between 0.54 – 1.59, 0.56 – 1.66 and 0.45 – 1.44 33 log CFU.mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. The combination treatment resulted in improved disinfection. This treatment effectively reduced microorganisms' numbers, including STEC to undetectable levels. Combination treatment also resulted in shorter UV exposure times. This was due to the improved physico-chemical quality of the water achieved by filtration.

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INTRODUCTION

Ultraviolet (UV) irradiation is a worldwide technology for water disinfection (Johnson *et al.*, 2010). It has gained popularity over the years, because of its effective inactivation of a wide range of microorganisms and little or no formation of harmful disinfection by-products (DBPs) (Mofidi *et al.*, 2002; Gayán *et al.*, 2013b; Turtoi, 2013). UV is effective against airborne pathogens, surface and water living bacteria, viruses and cyst formers (Johnson *et al.*, 2010) and has been used to treat drinking, irrigation, and wastewater (Johnson *et al.*, 2010; Turtoi, 2013; Vergine *et al.*, 2015; Ottoey *et al.*, 2016). Nevertheless, the effectiveness of this technology depends on certain important parameters such as water clarity (Johnson *et al.*, 2010). It is widely reported that the efficiency of UV irradiation decreases when the amount of suspended and dissolved particles in the treatment medium increases (Teksoy *et al.*, 2011; Gayan *et al.*, 2012; Turtoi, 2013; Jones *et al.*, 2014). These particles absorb UV light, thus, blocking microorganisms from the UV light (Jones *et al.*, 2014). The ideal water for UV treatment, therefore, should have minimal dissolved substances, turbidity and suspended solids, and also be low in organic compounds (Edstrom, 2011). In this regard, combining UV irradiation with a physical method such as filtration would overcome the limitation of highly turbid water. Filtration is a physical process that separates suspended and colloidal particles from water (Huisman & Wood, 1974). By reducing the load of suspended material present in the water, filtration would enhance exposure of microorganisms to UV light and subsequently lead to greater inactivation (Pettit, 2014).

Over the years, the use of biochar filtration has gained popularity in the water treatment industry, mainly because of its effectiveness in the removal of organic and inorganic contaminants from water (Ahmad *et al.*, 2017; Barancheshme & Munir, 2018; Van Rooyen, 2018). Biochar filtration is generally made up of an active charcoal derived from the pyrolysis of carbon-rich biomass (Barancheshme & Munir, 2018). It is a porous material that is rich in mineral elements and a large specific surface area (Dalahmeh, 2016). The main treatment mechanism of biochar is sorption (Barancheshme & Munir, 2018). Besides physical filtration through the biochar, an active biofilm develops and attaches to the biochar particle surfaces and mineralises organic matter from the water (Dalahmeh, 2016). The aim of this study was to, therefore, investigate the potential of pine biochar filtration as a pre-treatment method

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in order to improve the physico-chemical properties of river water including improving the UVT% of the water. A higher UVT% indicates better UV penetration. This could ultimately improve the UV disinfection efficacy against microorganisms. The following studies were conducted: first, the effect of pine biochar filtration in improving the physico-chemical properties of the river water was investigated; then the disinfection efficacy of the LP UV irradiation and pine biochar combination treatment was tested against the natural microbial population occurring in river water sampled from the Plankenburg River, Stellenbosch.

MATERIALS AND METHODS

General materials and methods

Water source

The Plankenburg River is approximately 10 km long and services various activities in the residential, industrial and agricultural sectors. The river system runs through the town of Stellenbosch. Next to the river is the Kayamandi informal settlement and an industrial area. In the Kayamandi area, some of the houses are made of bricks with in-house water connections and flush toilets, while the majority are backyard shacks and informal dwellings. The river serves as a source of irrigation water to farmers in the agricultural areas both up- and downstream from the settlement (Paulse *et al.*, 2009). It has previously been reported that the river carries very high *E. coli* loads (Paulse *et al.*, 2009; Ndlovu *et al.*, 2015; Olivier, 2015). This and the presence of Shiga-toxin producing *E. coli* was confirmed in Chapter 4 of this thesis.

Sampling method

The Plankenburg River was sampled four times during a ten-day trial conducted in the month of June 2018. Sampling was done every third day starting on day 1. The samples were collected using a sampling rod containing a sterile 1 L beaker and transferred to a sterilised 2 L bottle that was transported to the laboratory for analysis. All samples were analysed within 6 h of sampling and each experiment was performed in triplicate.

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Physico-chemical analysis

The Physico-chemical parameters (alkalinity, conductivity, chemical oxygen demand (COD), turbidity, total suspended solids (TSS), total dissolved solids (TDS), UVT%, volatile suspended solids (VSS), and pH) of river water samples were analysed. Alkalinity was determined by titrating 0.1 N H_2SO_4 into 20 mL of sample to reach a pH of 4.3. To measure the COD value in $mgO_2.L^{-1}$, a Spectro-quant Nova 60 COD Cell Test Kit (Merck Millipore, South Africa) measuring in the range between 10 – 150 $mgO_2.L^{-1}$ was used. A portable HI 8733 conductivity meter (Hanna Instruments, USA) was used to measure conductivity in $mS.m^{-1}$. An Orion AQ3010 Turbidity Meter (Thermo Scientific, USA) was used to determine the turbidity of river water in Nephelometric Turbidity Units (NTU). Solutions of known turbidity were used to verify that the instrument was calibrated. A portable pH meter (WTW, Germany) with a combination electrode was used, according to the manufactures instructions to determine the pH of river water samples. Known buffer solutions of pH 4.01 and pH 7.00 were used to standardise the equipment. A T254 UV Transmission % Photometer (Berson, Netherland) was used to determine the UVT% of river water samples. Distilled water was used for calibration, representing a UVT of 100%. Total dissolved solids (TDS) content of the water was determined using a total dissolved solids (TDS)-3 meter (HM Digital) that measures the total amount of mobile charged ions found in the water. TSS and VSS were gravimetrically determined at 105^{o}C and 550^{o}C, respectively, using Standard Methods (APHA, 2005).

Microbial analysis of river water samples

Enumeration of bacteria

Standard plating method was used for the enumeration of bacteria (*Enterobacteriaceae*, total coliforms and *E. coli*). Dilution series (10^{0}-10^{-6}) were prepared according to the South African National Standards (SANS) method 6887-1 (SANS, 1999) in 9 mL of Ringer's solution. Aliquots of 1 mL of each dilution were then transferred to duplicate Violet Red Bile Glucose (VRBG) agar (Merck, South Africa) plates for the enumeration of *Enterobacteriacea*e, and Chromogenic coliform (Oxoid, South Africa) agar for the simultaneous enumeration of total coliforms and *E. coli*. All plates were inverted and incubated at 37°C for 24 h, after which colonies between 25-250 were counted. Each experiment was performed in triplicate.

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Detection of Shiga toxin producing E. coli

Shiga toxin producing *E. coli* was detected using the DuPont™ BAX System real-time PCR assay (Hygiena) that detects the (*stx* and *eae*) genes. Detection was carried out according to the procedure described in the BAX System User Guide. In brief, 100 mL of water sample was filtered through sterile cellulose nitrate membrane filters with a pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa). The filters were then transferred to 20 mL of sterile buffered peptone water (Oxoid, South Africa) which was incubated at 37ºC for 24 h. Following incubation, 20 µL of the enrichment was transferred to 200 µL of prepared BAX System lysis reagent in cluster tubes. Lysis was performed by heating the tubes for 20 min at 37ºC and 10 min at 95ºC, and then cooling tubes to 4ºC for at least 5 min. Shiga toxin producing *E. coli* analysis was then performed by transferring 30 µL of the lysate to PCR tubes containing tablets. The PCR tubes were subsequently loaded into the BAX System Q7 instrument. A process involving amplification and detection was then run according to the procedure described in the BAX System User Guide. The analysis was carried out using software version 3.2 for standard assays.

Pine biochar filtration

The filtration columns (length in diameter) were made up of sterile glass with open ends. The bottom end was closed with sterile glass wool that was kept in place by a sterile metal mesh. Two different filter materials; (a commercial pine biochar and silica sand) were used in the construction of the columns. The bottom layer of the filtration column was made up of a 100 g layer of silica sand, with a grain size of 0.6 –1.2 mm. Subsequently, a 2 L layer of pine biochar with grain size of 0.6-1.2 mm was added. In order to prevent floating of the biochar, another 200 g silica sand layer with a particle size of 0.6–1.2 mm was added on top (Figure 1) (Tanino Febbraio, 2018, Food Science Department, Stellenbosch University, Personal communication).

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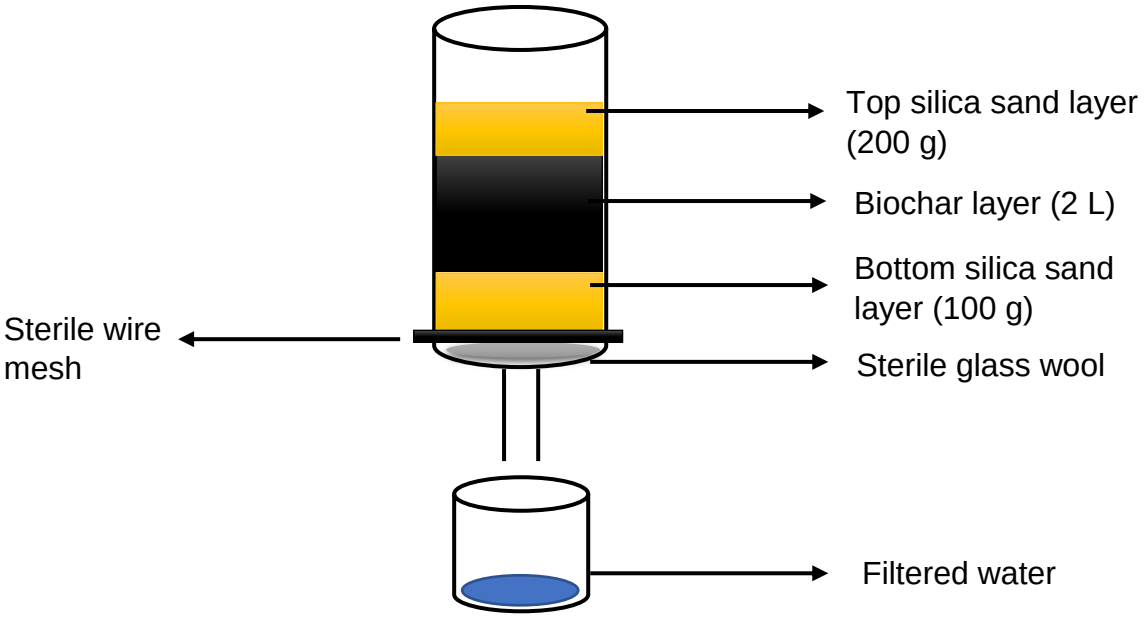

Figure 1 Schematic diagram of the laboratory-scale pine biochar filters used for testing river water treatment effects

Ultraviolet disinfection

Ultraviolet irradiation experiments were performed using a standard collimated beam device, containing a low-pressure mercury vapour lamp (Berson, The Netherlands), producing predominantly 254 nm wavelength UV irradiation. Before UV exposure, the UV lamp was turned on for at least 10 min to ensure a uniform lamp output. A glass beaker (250 mL) containing 100 mL of water sample was placed under the collimated beam and continuously stirred with the aid of a magnetic stirrer bar and plate. The UV dose, which translates to UV exposure time, was calculated as described by Hallmich & Gehr (2010).

$$I(\text{avg}, \lambda)\ (mW.\ cm^{-2}) = I0\lambda\ [\frac{1-\ e\text{dln}(\text{UVT}(\lambda))}{-\text{dln}(\text{UVT}(\lambda))}]\ [1]$$

$$Desired\ dose\ (mJ.\ cm^{-2}) = Average\ intensity\ (mW.\ cm^{-2}) \times Exposure\ time\ (s)\ [2]$$

In the equation above, I (avg, λ) is the average intensity of UV light over the sample depth, d; UVT(λ) refers to the UV transmission at wavelength, λ, determined using an optical path length of 1 cm; I0(λ) is the intensity of UV light measured at the surface of the sample

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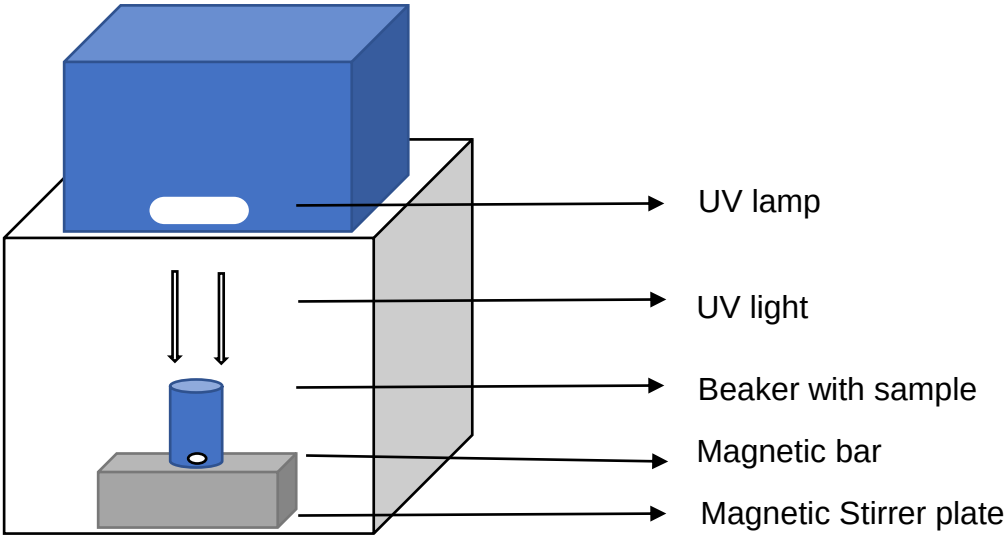

Figure 2 Representation of UV bench-scale irradiation used in this study

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Research design

To evaluate the potential of pine biochar filtration and UV irradiation combination treatments to treat microbiologically contaminated river water, the effectiveness of the two treatments were studied. The experimental design of this study is presented in Figure 5.

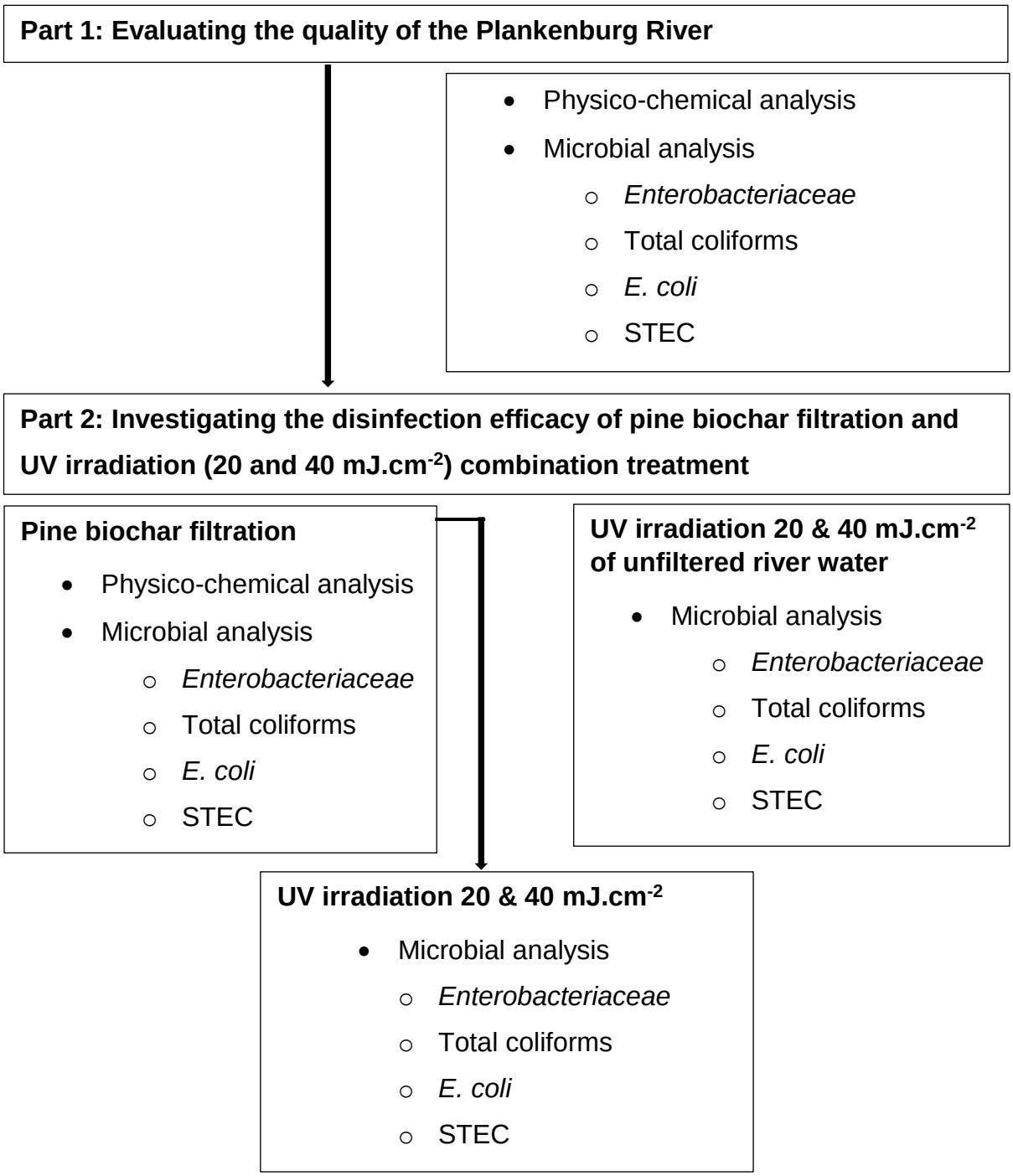

Figure 5 Flow diagram of experimental design followed in this study

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Part 1: Investigating the quality of the Plankenburg river water

The initial quality of the Plankenburg River water was determined by evaluating both the physico-chemical and the microbiological characteristics of river water before disinfection treatment. The physico-chemical parameters (alkalinity, COD, conductivity, turbidity, TDS, TSS, UVT %, VSS and pH) were measured according to procedures described in the general materials and methods section. *Enterobacteriacea*e, total coliforms and *E. coli* were enumerated according to the standard plating methods as described in the general materials and methods section. The presence of STEC was investigated using the DuPont™ BAX System Real-Time PCR assay (Hygiena)

Part 2: Disinfection efficacy of pine biochar filtration and UV irradiation combination treatment

To evaluate the disinfection efficacy of pine biochar filtration and LP UV irradiation combination treatment, the effectiveness of the treatments was evaluated alone and in combination.

Pine biochar filtration

Untreated river water was filtered through biochar filter columns (Figure 1), over a period of ten days. However, sampling and analysis of filtered water were only done every third day resulting in a total of four trials starting from day one. This was to evaluate the performance of the filters over time. Research has shown that the performance of some filters improves as filtration progresses. This is due to the possible formation of a biofilm that allows adhesion of contaminants on the filter (Dalahmeh, 2016). Physico-chemical parameters (alkalinity, COD, conductivity, turbidity, TDS, TSS, UVT %, VSS and pH) were analysed following filtration. This was done to evaluate the potential of pine biochar filtration in improving the physico-chemical parameters of river water. Microbial analysis was also done after filtration, using standard plating methods (*Enterobacteriaceae*, total coliforms and *E. coli*) and the DuPont™ BAX System Real-Time PCR assay (Hygiena) that detects STEC (*stx* and *eae*) genes.

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Ultraviolet irradiation

A bench scale collimated-beam device was used according to the procedure described in the methodology section to perform UV experiments. In this case, 100 mL of water samples in sterile 250 mL glass beakers were exposed to UV light at UV dose 20 & 40 mJ.cm^{-2}. *Enterobacteriacea*e, total coliforms and *E. coli* were enumerated following UV irradiation at dose 40 mJ.cm^{-2} and STEC were detected after UV dose 20 & 40 mJ.cm^{-2}.

Pine biochar filtration/UV irradiation combination

To evaluate the potential of combining UV irradiation and pine biochar filtration for the reduction of microbial loads in river water prior to irrigation, pine biochar filtered water was exposed to UV irradiation at two UV doses (20 & 40 mJ.cm^{-2}). Following this, *Enterobacteriaceae*, total coliforms and *E. coli* after UV dose 40 mJ.cm^{-2} were enumerated while STEC was detected after UV dose 20 & 40 mJ.cm^{-2}. Results were compared to those of the stand-alone treatments to determine the effectiveness of the respective treatments.

RESULT AND DISCUSSION

Investigating the quality of the Plankenburg river water

The quality characteristics of the Plankenburg River water were evaluated before disinfection treatment in four trials. The microbiological and physico-chemical properties that were analysed are summarised in Table 1.

Results show high levels of *Enterobacteriaceae*, total coliforms and *E. coli* present in the Plankenburg River on all four trial days. The population size of bacteria ranged between 4.17 – 6.01 log CFU.100 mL^{-1}, 4.07 – 5.95 log CFU.100 mL^{-1} and 3.75 – 4.94 log CFU.100 mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. For *E. coli,* these detected levels exceeded the recommended limit set for irrigation water (≤ 3 log CFU.100 mL^{-1}) (DWAF, 1996). Similar, but slightly higher *E. coli* counts were reported in Chapter 4 of this thesis: *Escherichia. coli* counts of up to 6.50 log CFU.100 mL^{-1} were observed. These results were observed a few months before the current results. The slight reduction in *E. coli* counts between the two studies is attributed to increased rainfall. Rain was recorded during sampling of the Plankenburg River in the current study. It might have, therefore, diluted the

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concentration of *E. coli* in the river, which might have resulted in lower counts. The results also correspond to those observed by previous researchers. Paulse *et al*. (2009) and Olivier (2015) reported *E. coli* levels of 6.50 and 6.41 log CFU.100 mL^{-1}, in the Plankenburg River, respectively. These indicate faecal contamination of the river probably through indiscriminate disposal of domestic wastes directly into the river (Paulse *et al.*, 2009). The results in this study, also indicate the persistence of the problem and highlight the health risks associated with water from this river system.

Although bacteria were present in river water on all four trial days, results show a clear variation in the microbial count of river water among these trials days. Water sampled in trial 3 had the highest microbial count of 6.01, 5.95 and 4.94 log CFU.100 mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively, followed by water sampled in trial 2 with 5.48, 5.36 and 4.52 log CFU.100 mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. Water sampled in trial 4 had the lowest microbial load for all trials of 4.17, 4.07 and 3.75 log CFU.100 mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli*, respectively (Table 1). The counts observed appear to be related to the change in weather patterns. Rainfall was recorded on the day of trial 1 and on the day before trial 4. These might have diluted the bacterial load present in the river, leading to a decrease in microbial levels in Trials 1 and 4 (Table 1). In addition to rainfall, other factors such as sporadic contamination might also have contributed to varying levels of microorganisms in river water.

Further evaluation of the river water also revealed variation in physico-chemical parameters of river water on the four trial days. Like the microbial counts, water sampled in Trials 2 and 3 showed the highest levels of some physico-chemical properties measured. The highest alkalinity (117.5 mg $CaCO_3.L^{-1}$),TSS (131.2 mg. L^{-1}) and VSS (22.8 mg. L^{-1}) were observed on the third trial day, while the highest turbidity (214 NTU) and TDS (239 $mg.L^{-1}$) values were observed in trial 2 (Table 1). High turbidity, which is a reflection of high levels of TDS, TSS and VSS in the sample (Johnson *et al.*, 2010), implies a high level of pollution of the Plankenburg River during trials 2 and 3. This was confirmed by the high microbial counts reported in trials 2 and 3 (Table 1). As expected, low UVT% values of 8.9 and 14% were observed for trials 2 and 3, respectively. This is because, low UVT% indicates that water has high levels of solids and organics (Gayán *et al.*, 2013a). Solids in rivers are often a result of sediments carried by the water, originating from natural and human activities such as soil erosion, urban run-off, and industrial effluents (Kolawole *et al.*,

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2011). Conductivity values were found to be similar on all four trial days. Values ranged between 0.26 – 0.37 mg.L^{-1}. Chemical oxygen demand results did not correspond to that of other physico-chemical parameters (Table 1). Comparison showed that water sampled in trial 1 had the highest COD value (39.3 mg.L^{-1}), followed by water from trial 2 (27.9 mg.L^{-1}) while trial 4 water had the lowest (12.45 mg.L^{-1}), which, unlike the microbial findings, suggested that water sampled in trial 1 was more polluted as COD determines the organic as well as inorganic content in the water (Rajiv *et al.*, 2012).

The pH of water is very important, because it adversely affects its use for irrigational purposes (Rajiv *et al.*, 2012). The pH values of water samples in this study were generally neutral on all four trial days (Table 1). The pH values ranged from 6.7 – 7.15. It is recommended that water pH should be in the range of 6.5 to 8.5 for agricultural irrigation purposes (DWAF, 1996). Because river water quality always varies depending on seasonal fluctuations of the environment, it was expected that the quality parameters of water would differ between the four different trial days.

Table 1 Quality parameters of water from the Plankenburg River on four different days

	Sampling days			
Quality parameters	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
Enterobacteriaceae (log CFU.100 mL^{-1})	4.77	5.48	6.01	4.17
Total coliforms (log CFU.100 mL^{-1})	5.07	5.36	5.95	5.07
E. coli (log CFU.100 mL^{-1})	3.94	4.52	4.94	3.75
Alkalinity (mgCaCO3.L^{-1})	95	85	117.5	61
COD (mg. L^{-1})	39.3	27.9	21.45	12.45
Conductivity (mS.m^{-1})	0.31	0.37	0.32	0.26
Turbidity (NTUs)	16.5	214	195	23
TDS (mg.L^{-1})	201	239	202	172
TSS (mg.L^{-1})	9.8	104.8	131.2	16.8
VSS (mg.L^{-1})	4	16.2	22.8	4.4
UVT%	47	8.9	14	53
pH	6.7	6.96	7.1	7.15

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Investigating the disinfection efficacy of pine biochar filtration and UV irradiation (20 and 40 mJ.cm^{-2}) combination treatment

Ultraviolet irradiation is widely recognised as a technology available to inactivate waterborne pathogens (Mofidi *et al.*, 2002; Teksoy *et al.*, 2011; Jones *et al.*, 2014). Contaminants such as suspended and dissolved solids have, however, been shown to reduce the effectiveness of UV irradiation by reducing the UV light that reaches the target organism (Teksoy *et al.*, 2011; Mounaouer & Abdennaceur, 2012). Filtration separates suspended and colloidal particles from water (Huisman & Wood, 1974). In this study, pine biochar filtration was used as a pre-treatment step to determine if it had any effect on the improvement of the physico-chemical properties of river water before UV disinfection.

The efficacy of pine biochar filtration in improving the physico-chemical parameters of water

The physico-chemical parameters of river water samples were measured in terms of alkalinity, COD, conductivity, turbidity, TDS, TSS, VSS, UVT% and pH before and after filtration through the pine biochar columns (Figure 1).

These results are presented in Table 2. In all trials, a significant reduction of COD, turbidity, TSS and VSS were observed following pine biochar filtration (Table 2). Trial 2 showed the highest reduction (174 NTU) of turbidity while trial 3 showed high efficiency in COD (19.25 mg.L^{-1}), TSS (113.4 mg.L^{-1}) and VSS (18.6 mg.L^{-1}) reduction (Table 2). Improvement in UVT% was also observed in all trials following filtration. Water samples from trials 2 and 3 had the most improved UVT % (Table 2). This could be due to the high reduction of turbidity, COD, TSS and VSS observed during these trials. Reduction in solids and organics allows UV light to pass through water unobstructed (Jones *et al.*, 2014), hence the improved UVT%. The improved UVT% resulted in reduced UV exposure times (Table 3). As shown, UV exposure times of water samples with low UVT% were longer than those of water samples with high UVT%. To achieve a UV dose of 40 mJ.cm^{-2} in trial 3, water samples with a UVT of 8.9% (before filtration) were exposed to UV light for 58:47 minutes, while water with a UVT of 62% (after filtration) was exposed to UV light for 15:01 minutes (Table 3).

The better performance of pine biochar filtration in trial 2 and 3 could not be explained. Contrary to this finding, Verma *et al*. (2017) found filtration to be effective

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at the start of filtration and decreased as the days went by. This is due to higher adsorption capacity of the filters at the start. As water treatment in filters proceeds, biofilm, solids and dead cells accumulate in the pores of the filter and decrease its porosity, which can progress to cause clogging of the pores and failure of the filter (Dalahmeh, 2016).

Unexpected results were observed with alkalinity, conductivity, TDS and pH (Table 2). In this case, an increase in these parameters was observed following filtration. The first trial showed the highest increase in alkalinity, conductivity, TDS and pH. An increase in pH values resulted in water being slightly alkaline following filtration (Table 2). Alkalinity is an estimate of the ability of water to resist change in pH upon addition of acid (Johnson *et al.*, 2010). Water with high alkalinity contains high concentrations of bicarbonate and carbonates (Ourimabah, 2011). This may cause calcium and magnesium to precipitate from the soil, which may affect plant growth (Ourimabah, 2011). In addition to nutritional deficiency of plants, water with high bicarbonates and carbonates can reduce the activity of pesticides by clogging the nozzles of pesticide sprayers and drip tube irrigation systems (Bailey & Bilderback, 2004). The desirable alkalinity limit range of water prescribed for irrigation purpose by (DWAF, 1996) is 30 -130 $mgCaCO_3.L^{-1}$. The increase in alkalinity of water following filtration in trials 1 and 2 can be attributed to water absorbing carbonates from the biochar filter material. Biochar has been shown to contain carbonates (Fidel, 2015). During trials 3 and 4, the alkalinity of the water is, however, within an acceptable range.

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Table 2 Physico-chemical parameters of river water measured before and after pine biochar filtration

	Trial 1		**Trial 2**		**Trial 3**		**Trial 4**	
Quality parameters	Before	Filtration	Before	Filtration	Before	Filtration	Before	Filtration
Alkalinity (mg CaCO3.L^{-1})	95	202	85	149.5	117.5	115	61	103
COD (mg.L^{-1})	39.3	25.1	27.9	15.75	21.45	2.2	12.45	4.8
Conductivity (mS.m^{-1})	0.31	0.51	0.37	0.42	0.32	0.42	0.26	0.32
Turbidity (NTU)	16.5	8.58	214	40	195	40	23	7.48
TSS (mg.L^{-1})	9.8	10.4	104.8	17	131.2	17.8	16.8	5.8
TDS (mg.L^{-1})	201	320	239	266	202	264	172	213
VSS (mg.L^{-1})	4	3.6	16.2	5	22.8	4.2	4.4	2.6
UVT%	47	88	8.9	62	14	63	53	85
pH	6.7	10.21	6.96	9.1	7.1	8.81	7.15	8.61

Table 3 UVT (%) and calculated UV exposure times (min) (at dose 40 mJ.cm^{-2}) of river water samples before and after filtration

	Before filtration		**After filtration**	
Trials	UVT%	Exposure time (min:sec)	UVT%	Exposure time (min:sec)
Trial 1	47	20:57	88	10:18
Trial 2	8.9	58:47	62	15:01
Trial 3	14	48:01	63	14:58
Trial 4	53	18:17	85	10:04

The effects of combining pine biochar filtration with UV irradiation on reducing the microbial load of river water

The benefits of combining disinfection methods are well documented (Gayán *et al.*, 2013a; Olivier, 2015; Zyara *et al.*, 2016). The effectiveness of pine biochar filtration/UV irradiation combination treatment in reducing microorganisms was evaluated in this study. Each treatment method was evaluated alone and in combination.

Table 4 shows the bacterial counts before and after each treatment. From the results, significant reductions in microbial counts were observed following the stand-alone treatments. Comparatively, UV irradiation showed better microbial reductions

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than pine biochar filtration on all four trial days. UV irradiation (40 mJ.cm^{-2}) resulted in undetectable levels of all bacteria when the initial microbial load was low (trials 1 and 4) (Table 4). When the water was more contaminated (trial 2 and 3), the bacteria counts in trial 2, decreased from 5.48 – 2.30 log CFU.100 mL^{-1}, from 5.36 – 2.34 log CFU.100 mL^{-1} and from 4.52 – 2.30 log CFU.100 mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. For trial 3, the bacterial counts decreased from 6.01 – 2.95 log CFU.100 mL^{-1}, from 5.95 – 2.58 log CFU.100 mL^{-1} and from 4.94 – 2.60 log CFU.100 mL^{-1} for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively (Table 4). These reductions resulted in final *E. coli* counts within the irrigation standard guideline limit of ≤ 3 log CFU.100 mL^{-1} of *E. coli* in irrigation water. As stated in Chapter 4 of this thesis, ≤ 3 log CFU in 100 mL^{-1} of irrigation water minimises the chance of pathogen transfer to fresh produce (Groves & Hulin, 2013).

Pine biochar filtration showed log reductions between 0.54 – 1.59 log, 0.56 – 1.46 log and 0.45 – 1.40 log for *Enterobacteriaceae*, total coliforms and *E. coli,* respectively. With better reductions being observed in trials 1 and 3 (Table 4). These log reductions were, however, unable to reduce the *E. coli* level to acceptable irrigation standard when the water was highly contaminated (trials 2 and 3). *Escherichia. coli* counts decreased from 3.94 – 2.78 log CFU.100 mL^{-1}, from 4.52 – 3.79 log CFU.100 mL^{-1}, from 4.94 – 3.54 log CFU.100 mL^{-1} and from 3.75 – 2.30 log CFU.100 mL^{-1} for trials 1, 2, 3 and 4, respectively. These imply that, pine biochar filtration cannot be used as a sole water disinfectant method, especially for highly contaminated water. Similar results were reported by Van Rooyen (2018). In his study, pine biochar filtration seldomly achieved more than 1 log reduction for any enumerated microbial groups (Total and faecal coliforms). The better performance of pine biochar filters in trial 1 can be attributed to the physical straining out of bacteria in the pores of the biochar during first-use of filtration. As water treatment in filters proceeds, organic matter accumulates on filters and form biofilms (Dalahmeh, 2016). While a thicker biofilm narrows pores in the filter, may contribute to the enhanced removal of bacterial particles by straining in narrow pores (Dalahmeh, 2016), excessive loading of the filters with high organic material may lead to failure of the filter by clogging (Verma *et al.*, 2017).

The combination treatment (pine biochar filtration/UV irradiation) achieved better reduction compared to the stand-alone treatments. As shown in Table 4, the combined treatment resulted in undetectable levels of all bacterial on all four trial days. The greater combined benefit was attributed to the contributions of each of the

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technologies. In trial 2, the *Enterobacteriaceae* log reductions were 3.18 and 0.70 log for UV irradiation and filtration, respectively. These log reductions combined, resulted in undetectable levels of bacteria. Although UV irradiation had the greatest effect in terms of log reductions, the effect of pine biochar on improving UVT% was also clear. The improved UVT% might have contributed to the greater inactivation achieved by allowing UV light to penetrate the water sample less obstructed (Jones *et al.*, 2014). Not only that, but the improved UVT% also contributed to better disinfection efficiency achieved following the combined treatment. As stated earlier, improved UVT% resulted in shorter UV exposure times (Table 3).

Table 4 Bacterial count (log CFU. mL $^{-1}$) of the Plankenburg River water before and after treatment.

		Treatments		
Enterobacteriaceae	**Before**	**Filtration**	**UV (40 mJ.cm^{-2})**	**Filtration/UV combination**
Trial 1	4.77	3.17	0	0
Trial 2	5.48	4.79	2.30	0
Trial 3	6.01	4.81	2.95	0
Trial 4	4.17	3.63	0	0
Total coliforms				
Trial 1	5.07	3.60	0	0
Trial 2	5.36	4.80	2.34	0
Trial 3	5.95	4.28	2.58	0
Trial 4	4.07	3.49	0	0
E. coli				
Trial 1	3.94	2.78	0	0
Trial 2	4.52	3.79	2.30	0
Trial 3	4.94	3.54	2.60	0
Trial 4	3.75	2.30	0	0

Table 5 Log reductions of filtration and UV irradiation as stand-alone treatments

	Enterobacteriaceae		**Total coliforms**		***E. coli***	
Treatments	Filtration	UV	Filtration	UV	Filtration	UV
Trial 1	1.59	-	1.46	-	1.16	-
Trial 2	0.70	3.18	0.56	2.02	0.73	2.22
Trial 3	1.20	3.06	1.66	2.36	1.40	2.33
Trial 4	0.54	-	0.58	-	0.45	-

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The efficacy of filtration/UV combination treatment against STEC

Pine biochar filtration and UV irradiation (20 and 40 mJ.cm^{-2}) were tested alone and in combination for the reduction of STEC. Table 6 shows the detection of STEC in river water samples before and after each treatment. As shown, STEC tested positive on all four trial days before treatment (Table 5). This also corresponds to results reported in Chapter 4 of this thesis, where STEC tested positive on all three trial days. These results suggest that the presence of STEC in the Plankenburg River is consistent and not sporadic. Similarly, Lamprecht *et al.* (2014) and Ndlovu *et al.* (2015) also detected pathogenic *E. coli* in the Plankenburg River water samples.

With regards to treatments, STEC was detected in three of four trials following filtration. This suggests that pine biochar filtration is not effective in inactivating STEC. Biochar filtration has been reported to be ineffective against the removal of pathogens (Dalahmeh, 2016; Van Rooyen, 2018). Following UV irradiation, no STEC was detected at a dose of 40 mJ.cm^{-2}, however, STEC was detected in trial 3 after a dose of 20 mJ.cm^{-2} (Table 6). These results imply that a UV dose of 20 mJ.cm^{-2} may not be enough to inactivate STEC especially if it is present in high concentrations. Contrary to these findings, results presented in Chapter 4 of this thesis showed that a UV dose of 40 mJ.cm^{-2} was not enough to inactivate STEC. The contradictions may be due to the concentrations of STEC present in the river. Again, the current results were observed during the rainy season which might have diluted the STEC concentrations in the river, which could easily be inactivated by low UV doses.

It was further observed that all samples tested STEC negative following the combined treatment at both UV doses (Filtration/20 mJ.cm^{-2} and Filtration/40 mJ.cm^{-2}) (Table 6). This illustrates the advantages of combining treatments in reducing pathogens. Again, this combined benefit is attributed to the contribution of each disinfection treatment. The effectiveness of combining treatments have been reported in water disinfection. Wang *et al.* (2011) found the combined effect of UV irradiation and chlorine to be more effective for the complete removal of bacteria in reclaimed water. Olivier (2015) also observed better microbial reduction following combined treatment of UV and hydrogen peroxide.

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Table 6 Disinfection efficacy of filtration and UV combination in STEC inactivation

Treatments	Before	Filtration	20 mJ.cm^{-2}	40 mJ.cm^{-2}	Filtration & 20 mJ.cm^{-2}	Filtration & 40 mJ.cm^{-2}
Trial 1	+	-	NT	-	NT	-
Trial 2	+	+	NT	-	NT	-
Trial 3	+	+	+	-	-	-
Trial 4	+	+	-	-	-	-

+= Positive, - =Negative, NT=Not tested

CONCLUSIONS

As stated, the disinfection technologies could not reduce the initial microbial loads including STEC to undetectable levels when used as stand-alone treatments. UV irradiation alone showed good reduction when the initial microbial counts were low. However, it could not effectively disinfect the water when the counts were higher. Ultraviolet irradiation also achieved good inactivation of STEC after a UV dose of 40 mJ.cm^{-2}. This inactivation could not be achieved when a UV dose of 20 mJ.cm^{-2} was applied. Ultraviolet irradiation also required longer UV exposure times when water was highly turbid. These results imply that UV irradiation is a highly effective water disinfection method and can be used as a sole disinfection technology. However, if greater inactivation is required, higher UV doses should be applied especially when water is highly contaminated or when considering pathogens such as STEC. Also, if the water is highly turbid, a pre-treatment step should be applied to improve the UVT% which will likely result in shorter contact times.

Pine biochar filtration alone exhibited low disinfection efficiency of all bacteria. Its disinfection could not reduce *E. coli* counts to meet the irrigation standard. This implies that this technology should not be used as a stand-alone technology to disinfect waterborne microorganisms. What was effective, however, was its effect on improving the physico-chemical parameters of river water. Pine biochar filtration had the effects of removing substances from water, which resulted in improved UVT%. The improved UVT% resulted in shorter UV exposure times. Pine biochar filtration can, therefore, be used as a pre-treatment step to improve disinfection efficiency.

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The sequential disinfection with UV and filtration worked synergistically to effectively reduce *Enterobacteriaceae*, total coliforms, *E. coli* and STEC. All bacteria were reduced to below detectable levels following combined disinfection. The UV exposure times required to achieve inactivation were also reduced. This study, therefore, highlights the efficiency of maximising the outputs of both disinfection processes and suggest the use of combination treatments, especially on highly contaminated water. Not only does it maximise output, but it also reduces the time and energy needed to deliver the same level of treatment.

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CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

General conclusions

Research has shown that surface waters are the major source of water for irrigation in many countries. Unfortunately, these sources can be highly contaminated with microorganisms due to pollution. Contaminated irrigation water has been linked to the presence of pathogenic microorganisms on fresh produce and has been implicated in many foodborne disease outbreaks (Steele & Odumeru, 2004; Jongman & Korsten, 2017). This is a major concern in South Africa, where some rivers used for irrigation carry high microbial loads (Paulse *et al.*, 2009; Olivier, 2015), which often exceed the guideline limit of 3 log CFU.100 mL^{-1} of *E. coli* in irrigation water recommended by the Department of Water Affairs (DWA) (DWAF, 1996). These high microbial loads in irrigation water present health risks, especially when used to irrigate fresh produce intended to be consumed raw. In order to minimise these potential risks associated with contaminated irrigation water, the water requires treatment prior to use for irrigation.

Various treatment methods including chemical and physical are available to disinfect water, with each method having its own advantages and disadvantages. On this account, UV irradiation has emerged as an effective water treatment method due to the advantages it has over other methods such as its ability to effectively inactivate pathogens while forming little or no disinfection by-products (DBPs) (Morita *et al.*, 2002; Mounaouer & Abdennaceur, 2012). The disinfection efficacy of UV irradiation, however, depends on many factors such as water quality and recovery potential of microorganisms. The overall aim of this study was, therefore, to evaluate the disinfection efficacy of low-pressure UV on river water with varying microbial and physico-chemical properties. The recovery potential of microorganisms was also evaluated.

The first part of the study investigated the LP UV disinfection efficacy of different UV doses (20, 30, 40, 50, and 60 $mJ.cm^{-2}$) against selected *E. coli* strains including Shiga-toxin producing *E. coli* (STEC) strains. From the results obtained, a target 3 log reduction was achieved for all strains at all UV doses. Higher UV doses resulted in higher log reductions, which implied that higher UV doses would result in greater

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microbial inactivation. The results also showed a clear variation in UV sensitivity among the *E. coli* strains at all UV doses. Compared to the ATCC strain (35218), the environmental strains (STEC 210 and F11.2) and a clinical STEC strain (STEC DP) were more UV resistant. This was true for all UV doses, indicating that certain strains are better adapted to withstand the effects of UV light. It was thus concluded that one strain cannot be used to represent a whole microbial species in UV irradiation disinfection optimisation studies as it might underestimate or overestimate the resistance of other strains. Based on these results, STEC 210 was selected and exposed to three different UV doses (20, 40 and 60 $mJ.cm^{-2}$) in both autoclaved river water and Ringer's solution to investigate the influence of water quality parameters. This strain was an environmental strain (originally isolated from game meat) that showed greater overall resistance to UV irradiation in the first study. The findings showed that water quality parameters such as turbidity did affect the UV disinfection efficiency. Longer UV exposure times had to be applied for highly turbid water in order to apply the same UV dose and achieve the same amount of inactivation as in less turbid samples. It became apparent from these findings that pre-treatment of the water before UV disinfection might be necessary to improve UVT%. This could reduce the UV exposure times required to achieve the same dose and ultimately improve UV disinfection efficacy.

The second part of the study investigated the LP UV disinfection efficacy of three different (Eerste, Krom and Plankenburg) river waters. The impact of water quality parameters and the recovery potential of microorganisms were also investigated. From this study, interesting conclusions were reached. The first was that Stellenbosch river waters vary in microbiological and physico-chemical quality. The Eerste River was the least polluted river and showed very low *E. coli* levels, which were well below the recommended irrigation guideline limit for fresh produce irrigation. The Department of Water Affairs (DWA) recommends a limit of 1 000 CFUs.100 mL^{-1} or 3 log CFUs.100 mL^{-1} of *E. coli* in irrigation water. The Krom River also conformed to the microbiological irrigation guideline limit. However, the once-off occurrence of STEC and consistent presence of ESBL producing *Enterobacteriaceae* despite the significantly low *E. coli* level in this river raised certain questions. This includes questions such as whether the Krom River was fit for irrigation purposes or not, and whether the microbial irrigation standard guideline of < 3 log CFU.100 mL^{-1} of *E. coli* is safe enough. It was also concluded from the Krom River results that low levels of

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indicator organisms (*E. coli*) do not necessarily indicate the absence of pathogenic organisms. The levels of indicators alone should, therefore, not always be considered as an indication of the presence or absence of other possible pathogens. The Plankenburg River was highly contaminated and carried *E. coli* at concentrations exceeding the guideline limit. The results also showed a consistent presence of STEC and ESBL producing *Enterobacteriaceae* in this river. It was concluded that water from this river could pose health risks and should therefore not be used for irrigation of fresh produce without prior treatment.

With regards to UV treatment, *Enterobacteriaceae*, total coliforms and *E. coli* could not be detected after UV disinfection at both doses (40 and 60 $mJ.cm^{-2}$) of river (Eerste and Krom) water with low initial microbial counts. A target 3 log reduction was observed for these microorganisms at both UV doses (40 and 60 $mJ.cm^{-2}$) following disinfection of water with high microbial loads (Plankenburg River). The 3-log reduction was able to reduce *E. coli* level in Plankenburg River water to meet the current irrigation guideline limit. These confirmed the capability of UV irradiation as an effective water disinfection method of indicator organisms. For STEC inactivation, however, a dose of 40 $mJ.cm^{-2}$ was inadequate. This implied that LP UV doses higher than 60 $mJ.cm^{-2}$ would be required to ensure adequate disinfection of serious pathogens such as STEC in river water.

The repair of microorganisms was observed following UV disinfection at both UV doses (40 and 60 $mJ.cm^{-2}$). Recovery was less than 1% at both doses. These still deserve further attention because, for water with high initial E. *coli* counts, 1% recovery can result in a microbial population size exceeding the stipulated guideline limit for water used for irrigational purposes. The repair was shown to be less at a higher UV dose (60 $mJ.cm^{-2}$). This implied that higher UV doses would result in less microbial repair.

It was also observed that the presence of suspended and dissolved particles in water reduced the UVT% which resulted in longer UV exposure times. These results confirmed that suspended and dissolved particles affect the UV disinfection efficiency.

In the final study, pine biochar filtration was used as a pre-treatment method in an attempt to enhance UV disinfection by improving water quality. Both treatment methods were evaluated alone and in combination to treat water from the Plankenburg River. Both treatments could not completely inactivate all microorganisms when used as stand-alone treatments. However, UV irradiation showed good microbial

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inactivation, while pine biochar filtration led to an improved UVT%. The results demonstrated the advantages of each method and concluded that pine biochar filtration can be used as a pre-treatment for UV disinfection of highly turbid water. When used in combination, all microorganisms including STEC were reduced to below detectable levels. The contact times of the treatment were also significantly reduced due to the improved UVT %. This study, therefore, highlights the fact that, the benefits of combining treatments does improve disinfection efficacy.

Recommendations for future research

Results from the first study indicated that UV resistance varied among the different *E. coli* strains tested. The environmental *E. coli* strains were more UV resistant than the ATCC strain. Again, this indicates that UV resistance varies between different organisms or even different species. It is, therefore, important to also consider the resistances of other important fresh produce-related pathogens such as *Salmonella* and *Listeria* in future UV optimisation studies, as opposed to only *E. coli*, and optimise UV treatments accordingly.

In the second study, it was observed that the Krom River carried low *E. coli* counts but showed the occurrence of a once-off STEC and persistent ESBL producers. Based on these, certain factors need to be considered. One key issue that needs to be addressed is to confirm the presence of STEC in the Krom River. From a practical point of view, it is important to further monitor this river. This will give an insight into whether the river harbours STEC consistently or not. Further, the microbial irrigation guideline only focusses on indicator organisms (*E. coli* < 1000 CFU.100 mL^{-1}). But other important pathogens linked to fresh produce may also be present in irrigation water. Another important recommendation is, therefore, to expand the microbial irrigation standard guideline to include monitoring of important food pathogens linked to fresh produce, such as STEC, *Salmonella*, *Listeria*, protozoan pathogens (*Cryptosporidium* and *Giardia*) and viruses. The Plankenburg River was highly contaminated and should, therefore, not be used for irrigation without prior treatment. UV treatment of river water indicated that a UV dose of 40 $mJ.cm^{-2}$ was unable to inactivate STEC. The use of higher UV (> 60 $mJ.cm^{-2}$) on STEC and other important waterborne pathogens should be further researched not only in terms of disinfection efficiency but also in terms of photo and/or dark repair. This would ensure the selection of dosages required to achieve enough inactivation of the most UV resistant

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waterborne pathogens including STEC. Results also showed recovery of microorganisms following UV disinfection at both UV doses. This study was, however, unable to determine whether the recovery was due to photo repair, dark repair or just regrowth due to extra nutrients. Based on this limitation, further investigation to determine the extent of photo-reactivation and dark repair by bacteria after exposure to UV light is therefore recommended.

Finally, the last study observed that pine biochar filtration improved UVT% of the water which led to improved disinfection efficacy of UV dose 40 $mJ.cm^{-2}$. Combining pine biochar filtration with different UV doses (< 40 $mJ.cm^{-2}$) in future studies should be further investigated. This could lead to the selection of an optimum combination treatment. What also needs to be addressed is the effects on UV/filtration combined treatment on photoreactivation and dark repair - whether pre-treatment (filtration) would minimise the occurrence and at which UV dose - filtration combination it would be minimised.

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